VOLUME LVII NUMBER 6

THE
BOTANICAL GAZETTE

*JUNE 1914*

# WINTER AS A FACTOR IN THE XEROPHILY OF CERTAIN EVERGREEN ERICADS[1]

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(WITH TWELVE FIGURES)

Of late years considerable attention has been drawn toward the apparently anomalous condition of several plants with obvious xerophytic modifications living in bogs with an apparently unlimited water supply. Many explanations of this apparent anomaly have been attempted. It was with a desire to obtain further knowledge upon the question that the author entered upon this piece of research work in the Botanical Department of the University of Michigan in the fall of 1910.

The work was carried on under the direction and supervision of Professor F. C. NEWCOMBE. To him I am greatly indebted both for the opportunity to work and for his stimulating criticism throughout the work. To Dr. H. A. GLEASON and to Dr. J. B. POLLOCK, both of the University of Michigan, I am also indebted for helpful conferences during the course of the work. To Mr. W. B. MCDOUGALL, of the University of Michigan, I am further indebted for the examination of material for the presence of mycorhiza. The nomenclature is that of the seventh edition of GRAY'S *Manual*.

[1] Contribution from the Botanical Laboratory of the University of Michigan, no. 136. The part of this work done during the summer of 1912 was carried on with the aid of a grant from the American Association for the Advancement of Science.

## General discussion

In order to maintain existence, it is necessary for an organism to fulfil the fundamental requirements of life; it must be able to take in food; it must be able to digest its food; it must be able to oxidize or otherwise rearrange its substance to obtain energy; it must be able to eliminate its waste products; and it must be able to perpetuate its kind. Further, it must be able to perform all these functions in its particular, individual environment. As these individual plants cannot migrate, they must be able to accommodate themselves to the changing environmental conditions or die. That they flourish from year to year in healthy condition is unquestionable evidence that they are able to cope with their environment. Their ability to invade genetically lower associations of plants indicates that they are thriving rather than just merely existing in their habitat.

Although a living plant is always the expression of the integration of environmental and hereditary factors, the most important single factor in the environment is the physiological water supply. The modifications of plant structure which lead to the conservation of the water supply are termed *xerophytic adaptations* or *xerophytic reactions*. The presence of xerophytic adaptations does not necessarily predicate that the amount of water used by the plant is relatively small, but that the ratio of the amount used to that which the plant obtains tends to become less than unity. Some so-called xerophytic plants use as much or more than ordinary mesophytic plants, as GROOM (**20**) found was the case with *Larix decidua*. They are xerophytic, however, because they cannot absorb a large amount of water in proportion to that which they could otherwise transpire.

This is particularly true in the summer, when plants have their transpiring organs. The loss of leaves during the winter is quite rightly regarded as a xerophytic adaptation. The bog ericads which were investigated, however, retain their leaves during the winter. This opens at once the question, are these plants xerophytes because of their summer or their winter environment? As it may be safely assumed that the evergreen habit is hereditary in these ericads, the reaction to the environment necessitates the xerophily.

It would seem, at first glance, that plants which grow in bogs, where there is an obvious physical water supply, would not be restricted in its use, but the various xerophytic adaptations argue for the conservation of water in the plant. This fact led investigators to ask why the plants could not make full use of the water present. Many answers have been attempted, and it seems quite likely that the true answer is a combination of the different reasons rather than any one. The problem presents an obvious result obtained from a bewildering mass of causes, whose interactions are not yet known.

The ability of peat bog plants to absorb water is limited on account of poorly developed, shallow root systems (FRÜH and SCHRÖTER **18**), low oxygen content of the water (DACHNOWSKI **9** and HESSELMANN **23**), low aeration (TRANSEAU **49**, DACHNOWSKI **10**, and FREE **17**), root excretions (LIVINGSTON, BRITTON, and REID **27**, and SCHREINER and REED **45**, **46**), bog toxins (LIVINGSTON **29** and DACHNOWSKI **9**, **10**, **11**), the necessity of mycorhizal fungi in some species, the low temperature of the soil water (KOSAROFF **25**, FRÜH and SCHRÖTER **18**, and especially TRANSEAU **49**), and biological processes rather than chemical differences in the soil (DACHNOWSKI **12**). Much stress cannot be laid upon the acidity of the soil, as has been done by SCHIMPER (**44**), because of the findings of later investigations. The acidity is very low and differs in different bog associations (TRANSEAU **49**). That acidity is a necessary factor in the soil for the growth of trailing arbutus (*Epigaea repens*) and of the blueberry (*Vaccinium corymbosum*) was most admirably demonstrated by COVILLE (**6**, **7**), who found that poor aeration was usually the real cause of poor growth and not acidity. Acidity, however, may be inimical to certain crops. SAMPSON and ALLEN (**42**) found that, as a rule, some of the common acids accelerate transpiration, and that weak solutions often produce as marked effects as strong ones.

The water absorbed is conducted up through the stems. A study of stem structure would show whether the ericads differ essentially from other bog shrubs. In either case the ability to conduct water must be adequate, as the plants thrive. From the stem the water passes into the leaves, where the largest

part of it is vaporized and passes out of the plant through the stomates.

Xerophytic responses may be classified into means retarding transpiration in the leaves or transpiring organs, absorption in the roots, checking transportation in the conducting tissue, or provision for an accumulation of water. In the peat bog ericads used during the course of this investigation, xerophytic response is very evident in the leaves, but is not accompanied by water storage tissue, which makes the xerophytic structure more necessary on account of the poorly developed root sytem. The evergreen habit, with its relatively large exposure of leaf surface, calls for greater activity of the root system throughout the winter, for transpiration still continues even when the thermometer is below zero. That means to reduce the loss of water are all the more necessary under winter conditions is obvious.

## Seasonal history of peat bog plants

### EVERGREEN ERICADS

During the winter the leaves of all of the evergreen ericads, *Chamaedaphne calyculata*, *Andromeda glaucophylla*, and *Vaccinium macrocarpon*, are upright, a position in which they receive a minimum of direct sunlight. The leaves are dark red or brown in color. With the coming of spring the old leaves curve outward or downward, resulting in an increase of the direct sunlight which they receive. At the same time the leaves become dark green in color. The season's growth of young leaves takes place soon after flowering. At first the young leaves are upright, but in a short time they bend outward. As soon as the young leaves are fully developed, the old leaves gradually drop off. In the case of *Vaccinium macrocarpon*, however, some of the leaves may be retained for two or three years. With the coming of the next winter, the leaves of these plants gradually bend up into an upright position and their color changes from bright green through dark green to shades of red and brown. The color changes begin at the margins of the leaves and work toward the midribs. In a mild winter the basal portion of midribs of *Chamaedaphne* may remain green the entire winter. *Vaccinium* and *Andromeda* are usually protected by a covering of

snow, but they exhibit these changes of position and color irrespective of that fact.

DECIDUOUS TREES AND SHRUBS

The principal trees are *Larix laricina* and *Acer rubrum*, and the commonest shrubs are *Aronia melanocarpa*, *Salix pedicellaris*, *S. discolor*, *S. sericea*, *Spiraea salicifolia*, *Betula pumila*, *Nemopanthes mucronata*, *Ilex verticillata*, *Gaylussacia baccata*, *Cornus paniculata*, *Cephalanthus occidentalis*, *Sambucus canadensis*, and *Rosa carolina*.

During the winter, bog trees and shrubs are leafless, which greatly reduces the transpiration. The snow that is present during the winter protects the root system and lower part of the stem from danger from excessively low temperature, but the upper parts of the trees and shrubs are not so protected. They must be able to resist water loss through their own modification. This is sufficient for the severest winters in southern Michigan. During the winter of 1911–1912, *Spiraea* was the only deciduous shrub to be killed down to the snow line.

With the opening of spring the buds swell and develop into branches bearing leaves which carry on the work of the season. A separation layer is formed upon the approach of winter at the base of the petiole, and by the time winter has set in the leaves have fallen.

HERBACEOUS PLANTS

The seasonal history of the herbaceous bog plants follows two general lines: the plant which has developed during the growing season may die down completely before winter, leaving seeds to reproduce it the following year, or it may die down to the ground and be vegetatively reproduced the following year from underground stems, bulbs, rootstocks, or buds. Any of these ways is an absolute xerophytic adaptation on account of winter conditions, but does not interfere with summer development.

## Structure of certain peat bog plants

ROOT SYSTEM

Without exception, all of the forms dealt with had a very shallow root system, which was usually very poorly developed. It is in direct contrast to that of the xerophytes of the desert (CANNON 4).

Two general types of roots could be separated, according to the presence or absence of mycorhizal fungi. Most ericads have mycorhiza, but none were found upon the roots of *Andromeda* or *Chamaedaphne*. In working over the material furnished him by the author, McDOUGALL found that *Andromeda* occasionally gave evidences of mycorhizal appearance, although further investigation failed to reveal its presence. Mycorhiza was found on *Larix laricina*, *Acer rubrum*, and *Vaccinium macrocarpon*, but was not noticed on any of the following plants: *Carex filiformis*, *Sagittaria latifolia*, *Eupatorium perfoliatum*, *Dulichium arundinaceum*, *Asclepias incarnata*, and *Aspidium thelypteris*.

The absence of mycorhiza on *Chamaedaphne* and these other plants demonstrates that it is not a necessary adaptation to the bog environment. The presence of resin deposits (TRANSEAU **49**) is often a noteworthy feature of the roots of bog plants. Root hairs were not observed, although TRANSEAU (**49**) found that in culture solutions, which were well aerated, normal roots with root hairs were produced in *Larix*.

During the summer, the roots of the bog plants, at least apparently, have an abundant water supply, although as a matter of fact the *Sphagnum* which surrounds the roots may be physiologically dry, even when apparently wet, on account of its great ability to soak up and retain water (FRÜH and SCHRÖTER **18**, and DAVIS **14**). In general, however, there is standing water beyond the ability of the *Sphagnum* to absorb, and therefore the bog plants have a supply to draw on throughout a normal season. Seasons of drought, therefore, would be the critical ones, and that of 1911 was a case in hand. In so far as could be observed, it did not appear that the ericads were suffering from lack of water even on the hottest and driest days. The leathery nature of their leaves makes it nearly impossible to tell whether the plants are wilting or not, even when herbaceous vegetation was obviously wilted. In the natural distribution of these plants, droughts are not sufficiently extreme nor of sufficient duration to dry out the *Sphagnum*. The great ability of *Sphagnum* to soak up and retain water localizes the water within reach at the expense of the surrounding area. During the summer this often results in the elevation of the water table

under the bog several inches above that of the surrounding country.

Taking into consideration the rarity of real drought conditions of long duration, it is evident that the root system in the bog habitat is able more than merely to maintain these plants within their normal range throughout drought conditions. The xerophytic adaptations of the transpiring organs, of course, materially aid by lessening the demand upon root absorption.

During the winter the ground is normally frozen. On account of the low position of bogs, they are more subject to early and late freezes than the surrounding country. Although the ground may be frozen, the covering of snow prevents the access of very low temperatures to the roots. In spite of the fact that the ground is frozen, it is evident from the continual water loss of the above-ground parts that some water is being absorbed by the roots, quite likely the water vapor evaporated from the ice into the spaces which become opened around the roots soon after the freezing of the ground.

At any of the temperatures at which roots were dug up (down to $-10°$ C.) it did not appear that any part of the plant was frozen. All parts were pliable to handling. The exposure of severed parts of *Chamaedaphne* for one-half an hour to $-25°$ C. resulted in freezing and loss of pliability. It was repeatedly noticed that the leaves which had been exposed to the severest weather of the winter, including a temperature of $-29°$ C., were dry, and cracked when bent. Later it became evident that these leaves had been killed. Beyond this simple test, whose limits of accuracy are not known, there were no suitable means of determining in the field whether the plant tissue was frozen.

## CONDUCTING SYSTEM

The conducting system in bog ericads consists of a very narrow ring of young xylem just outside the old wood (fig. 1). There is a very striking similarity in the appearance of the cross-section of the three ericads studied. The type of stem represented in bog ericads is strikingly different from that of other bog shrubs in the relatively smaller amount of conducting tissue and in the smaller

lumina of its cells. In this respect bog herbs are all different from the bog ericads also. Among the bog shrubs the ericad type stands out distinctly from all the other shrubs, there being far less difference between the structure of any two ericads than between an ericad and any other bog plant.

Just how the water is conducted from the roots to the transpiring organs is not a closed question. For a discussion of it the reader is referred to the literature, particularly COPELAND (5), DIXON (16), OVERTON (37), RENNER (41), SCHERMBEEK (43), and BABCOCK (1). The results of this investigation show that the fundamental control of rate of conduction is exercised by transpiration. An increase of transpiration always means an increase of conduction, and a decrease in transpiration means a decrease in conduction, though not always in the same proportion. Absorption and conduction are more closely related to each other than to transpiration, and they are more closely related to the turgidity of the cells than is transpiration. By reducing the turgidity of the cells transpiration exercises a control over the other two.

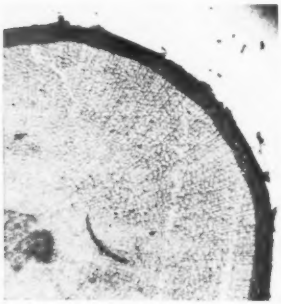

FIG. 1.—Photomicrograph of the stem of *Chamaedaphne calyculata:* the dark outer ring is cortex, just within it is the medium colored young xylem, then the light colored wood, and in the center the pith.

## UTILIZATION SYSTEM

Although the external appearance of the leaves of various peat bog plants is very different, the general internal structure is more nearly similar, and that of the various ericads is still more alike. Several well marked xerophytic adaptations are present, notably the strongly cuticularized epidermis, absence of stomates on the upper surface, a well developed palisade layer one to three cells thick, frequently sunken stomates, and coatings of wax, bloom, hairs, or scales. Mechanical tissue is present and accounts for the suppression of the ordinary symptoms of wilting. Usually the

leaves are at least slightly revolute, those of *Andromeda* and *Salix candida* strongly so. The leaves are usually dark green in color, but often reddish at the beginning and close of the vegetative season. The abundant presence of cutin in the evergreen ericads as an efficient xerophytic adaptation against loss of water at all times, but especially in winter, has been brought out by WIEGAND (**51**).

A considerable amount of water is transpired by many bog plants, and the loss may be as great as or greater than that from mesophytic plants of the same vicinity. This suggests that it is the maximum rate to which the plant may be subjected rather than the amount of water lost that is the important consideration. If the amount of food material is correlated with the amount of water lost, there would have to be considerably more water absorbed in the bog habitat, as it is notably deficient in available mineral food material (TRANSEAU **49**). Plants that normally grow in non-bog conditions, as white pine and black spruce, when growing under bog conditions are much dwarfed and stunted, and their leaves exhibit very pronounced xerophytic modifications, so much so that these plants growing in bogs have received specific designation.

Some plants, as *Populus tremuloides* and *Poa pratensis*, that may grow in either bog or mesophytic soil, do better in the latter situation and always exhibit a pronounced xerophily in the bog soil. Some plants demand bog conditions and even then have xerophytic modifications, as COVILLE (**6**) demonstrated in the case of the blueberry.

During the growing season, all of the bog plants have their transpiring organs, but the great majority do not retain them during the winter. In every case where leaves are retained, their winter position is different from their summer one. The winter position is usually upright, but in the evergreen conifers the leaves are more closely appressed to the twig. The young leaf as it comes out in the spring is also upright and remains so at least as long as it is tender. The fact that the dark upper surface is innermost when the leaves are upright serves to protect it by reducing the amount of radiant energy absorbed, which would raise the temperature of the mesophyll cells and lead to greater loss of water. The under surface is already well protected. The upright, upper surface to upper

surface position of the leaves during winter is really a xerophytic modification, reducing the amount of radiant energy absorbed at a time when it would be needlessly dissipated in increase of water loss, which the absence of phytosynthesis and the closure of the stomates does not occasion. Leaves which develop upon *Chamaedaphne* in the *Larix* association are much less xerophytically modified. They are much more subject to winter killing.

The loss of water by the leaves exercises a twofold function. The excess of radiant energy absorbed and not used in photosynthesis could easily raise the temperature of the leaf to the death point during hot waves, were it not dissipated in vaporizing water. DARWIN (13), through the use of a resistance thermometer, demonstrated that, with the check to transpiration that comes with induced closure of the stomates, the temperature of the leaf rises. Normally this higher temperature would not occur, for the excess of radiant energy being used to vaporize water causes a lowering of temperature. The loss of water in the leaves maintains a stream of water from the roots up. This is necessary for the removal of the products of respiration (BABCOCK 1) and for the lifting of the absorbed mineral material to the leaves. Water is also necessary in photosynthesis.

As LIVINGSTON (30) puts it: "The total amount of transpirational water lost from a plant, for any given period, may be considered as a summation of the effects of the evaporating power of the air and of the radiant energy absorbed throughout the period, modified by certain secondary effects of these conditions and certain responses to other conditions." The ratio of the water income to that of the removal must not fall below unity for any considerable time in plants which are not water-storing. Quoting again from LIVINGSTON (31): "The really crucial question with regard to any soil . . . . is . . . . *at what rate, and for how long a time, can it deliver water to a unit area of a water-absorbing surface?*" That water is supplied in sufficient quantities during the most extreme conditions of summer that obtain in nature in this region is evident from this investigation. The opposite statement is true for winter, namely, that in very severe winters the removal of water from the exposed parts of certain plants is so in excess of the supply that too

thorough drying and therefore death result. This same process has already been shown by KIHLMAN (**24**) to be the cause of the arctic tree line.

That the water supply for ericads in Michigan peat bogs is actually ample to their needs is clearly demonstrated by experimentation upon potted plants, for even under the very extreme evaporating power of the air on July 5, 1912, the maximum rate of transpiration was contemporaneous with the maximum evaporating power of the air. Conditions of atmospheric evaporating power in Michigan are never as high as those of Arizona, where LLOYD (**32**) found that the fall in the rate of transpiration in ocotillo (*Fouquieria splendens*) occurred before that of the maximum evaporating power of the air. Whether these results may be the true expression of the behavior of rooted plants may be open to question, as LLOYD used cuttings to experiment with. It was found in the present investigation that on days of extreme evaporating power in Michigan a decline in the transpiration rate in advance of the time of maximum evaporating power of the air did actually occur in cuttings, but was not exhibited in potted plants of the same species. Such a check in transpiration is occasioned by what LIVINGSTON and BROWN (**28**) have termed "incipient drying," in the course of which the evaporating menisci have retreated into the pores of the cells, thereby not only decreasing the amount of the exposed surfaces, but also greatly increasing the surface tension of these evaporating surfaces, which decreases the vapor tension and consequently the rate of vaporization (RENNER **39, 40,** and PATTEN **38**). The increase in the concentration of cell sap which accompanies this check in water removal further retards vaporization. A very serviceable pictorial presentation of the matter is given by MACDOUGAL (**34**).

The recent work of some investigators seems to withdraw the foundations from the theory of the efficient function of the stomates as the regulators of transpiration (LLOYD **32, 33,** and others). That closed stomates are efficient means of lowering transpiration has been demonstrated by many authors (BURGERSTEIN **3**, and DELF **15**). The closure of the stomates of evergreen plants during winter, which has been demonstrated by several investigators,

especially STAHL (**47**), is an important factor in reducing transpiration at that season, when the water intake is at best very low. The work of LLOYD (**32**) on *Fouquieria* led him to conclude that the capacity of the diffusion of the stomates was well in excess of what would be required for the greatest observed transpiration rate. F. DARWIN (DELF **15**), in a preliminary account before the British Association, concluded that if the stomates can be observed by a sufficiently delicate method, the stomal movements will be found to correspond closely with changes in the rate of transpiration caused by alteration in external conditions.

In the present investigation, in which the method of relative time of penetration of an oil was used to indicate the condition of the stomates, there was no evidence that the stomates exercised a "closely regulatory" function. The stomates opened in the morning, in general in the diffused light of dawn, but the rate of transpiration showed no sudden rise, but rather kept proportional to that of the evaporating power of the air. In the afternoon the stomates did not begin to close until after the beginning of the decline in transpiration. This was true both in potted plants and in cuttings properly cared for on days that were not extreme. In many cuttings the closure of the stomates seemed to be due to the shock of cutting rather than to any excessive water loss. Almost all of the wilted plants had their stomates closed, but in dried leaves the stomates were open.

The experimentation on plants in the field led to the conclusion that the stomates were open during the hours of sunshine, and that, although the opening of the stomates preceded the rise in transpiration in the morning, the decline in transpiration set in in the afternoon before the beginning of closing of the stomates. The rate of transpiration sank more quickly to a lower level than the time it took the stomates to close could possibly account for.

## Experimentation

### MATERIALS AND METHODS[2]

Throughout the study of this problem an experimental method was used which yielded numerical data. The experiments were carried on upon bog plants, principally *Chamaedaphne calyculata*

[2] Cf. BURGERSTEIN **3**.

(L.) Moench, obtained from First Sister Lake, a little west of Ann Arbor, Michigan, and at Mud Lake in the northern part of Washtenaw County, Michigan.

Toward the close of autumn in 1910 and 1911, plants of the evergreen ericads were potted and kept outdoors under prevailing conditions. During the middle of winter their transpiration was determined by successive weighings on a beam balance sensitive to 0.002 gram. The pot was inclosed by an aluminum shell (devised by GANONG) closed at the top with rubber dam and sealed with wax, whereby the water loss was limited to the plants, as controls repeatedly demonstrated. Readings of weight to 0.01 gram, temperature by mercury thermometer and thermograph, relative humidity by wet and dry bulb thermometers, and general conditions of the weather were recorded.

A number of pottings of ericads and other plants were made at First Sister Lake, June 1, 1912, the plants allowed to develop under bog conditions, and experimented upon the first week of July 1912.

By far the greater part of the work, however, was conducted with cuttings. These were made from the plants at First Sister Lake, immediately cut under water in jars and brought into the laboratory where they were again cut under water. The cuttings were then set up in two-holed rubber corks in bottles of distilled water and sealed with vaseline. A thermometer inserted in the other hole of the cork gave the temperature of the water. Work with controls proved that the apparatus was water-vapor tight. Usually about one-half an hour was allowed for adjustment before measurements were commenced. Weighings were made at intervals of one, two, or more hours, according to the purpose of the experiment. Such experiments were seldom carried over 24 hours, except for special reasons. The day of 24 hours of 100 "minutes" each was used in recording experiments, because of its obvious convenience.

Some plants which were transplanted into the greenhouse in *Sphagnum* did so poorly that no experiments were made upon them. Shoots that developed on cut twigs kept in water in the laboratory or greenhouse seldom lived more than a couple of weeks, and as their internal structure was not normal, no experiments were performed upon them.

It was found that different *Chamaedaphne* plants from the *Chamaedaphne* association (fig. 2) transpired at virtually the same rate during the same experiment. Consequently, that plant was taken as the basis for all comparisons and was included in practically every set of experiments.

With the close of the experiment the leaves were detached, placed side by side on white paper, covered with a thin piece of glass, and their outlines traced with a polar planimeter, by which means the leaf area was obtained. After a little practice with this instrument it was found that successive determinations of the same set of leaves did not vary by as much as 0.3 per cent. Accordingly the average of two determinations was used throughout the work. When stomates were also present on the upper surfaces (the rare exception in the plants used), the area thus obtained was doubled.

With the data so recorded, the results of each experiment were calculated with the aid of a slide rule to a standard basis, the rate of transpiration in grams per hour per 100 sq. cm. of leaf surface. These results were plotted on cross-section paper and the comparison made. As more than one determination for each plant was made, the resulting curves should approach a general similarity. Under the same conditions the similarity of the graphs was striking. Through dissimilarity of the resulting graphs, it was possible both to demonstrate the effect of change of experimental conditions and to weed out aberrant plants. As the greater part of the work was concerned with relative values, the continued reappearance of the same result in the graphs was taken to uphold the contention and no contention not thus uniformly upheld is presented in this paper.

During a part of the year 1912 the volume of the leaves was also determined by ascertaining the amount of alcohol they displaced. Alcohol was used in place of water on account of the large amount of air coating the leaves submerged in the latter. The results were calculated on the basis of water loss per hour per 1 cc. of volume. This was done in order to incorporate the results obtained from plants, the difficulty of determining the leaf surface of which would otherwise have rendered it virtually impossible. The correlation

of volume and leaf surface was irregular. With leaves of about the same size and thickness, the amount of water loss varied proportionally. In plants of *Chamaedaphne* from different plant associations, where both size and thickness of the leaves varied (fig. 2), it did not usually appear that volume was any constant function of the leaf area.

While it is recognized that the measured leaf area is not the area of the water-losing mesophyll cells, it is believed that it furnishes

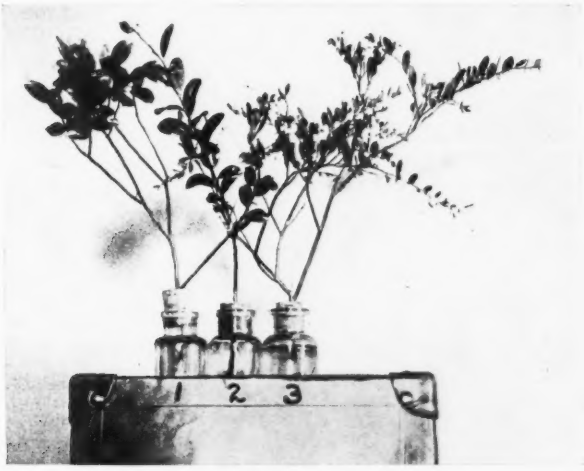


FIG. 2.—Twigs of *Chamaedaphne calyculata*, showing the character of the leaves developed in the *Larix* association (bottles 1 and 2) and in the *Chamaedaphne* association (bottle 3), at First Sister Lake; April 29, 1911.

a satisfactory basis of comparison attained without the excessive difficulty that would attend the determination of the actual area of the surface abutting upon the intercellular spaces. The leaf surface, moreover, is the area through which the diffusion into the outer air takes place.

To obtain a knowledge of the evaporating power of the air an open dish of water was run with several of the experiments.

At the close of many of the experiments a section of the smallest part of the stem below any transpiring organs was cut out,

preserved in glycerin-alcohol, later sectioned, and stained with iodin green to show the area of the conducting system.

By means of the lithium nitrate method, which consists of cutting off the lower ends of leafy stems under a 0.5 per cent aqueous solution of $Li(NO_3)_2$, allowing the stems to take up the solution, removing after certain intervals, and cutting immediately into 1 cm. lengths, testing these pieces in the spectroscope for the presence of lithium, a knowledge of the rate of conduction under different conditions was obtained. This method only approximates the rate of conduction in rooted plants, which could not be used because of inability to know when the lithium nitrate would be absorbed by the roots.

The difficulty of examining the stomates of *Chamaedaphne* by the ordinary method of stripping and the consequent uncertainty of its results with this species led to the abandonment of work on stomates until the publication of a new method (MOLISCH **35**) opened the way for experimentation upon this pertinent question. The "infiltration method," as it is called, depends upon the fact that when a leaf is wetted with a penetrating liquid, such as absolute alcohol, xylol, or turpentine, and held up to the light, it becomes translucent as soon as the liquid has penetrated the leaf. The relative time that it takes the leaf to become translucent after the application of xylol indicates whether the stomates are open or closed, because the more the stomates are open, the easier and quicker will the liquid penetrate the tissue and the sooner will it become translucent. A "normal" time must be determined for each species upon which to base deviations. Xylol was used throughout the present work, and the results were checked up with absolute alcohol and turpentine. As the method is so very simple, it can be employed in the field and several determinations made each time. The results were remarkably uniform.

## EXPERIMENTATION DURING THE WINTER

### *Transpiration*

During winter the transpiration of the plants of the region is reduced to a serviceable minimum which, however, is not zero (cf. KUSANO **26**). For herbaceous plants the minimum is lower

than for shrubs and trees. No experimentation was performed upon herbaceous plants during the winter, for it is known how exceedingly small is the amount of water loss from seeds, and as the vegetative means of reproduction employed by other herbs are

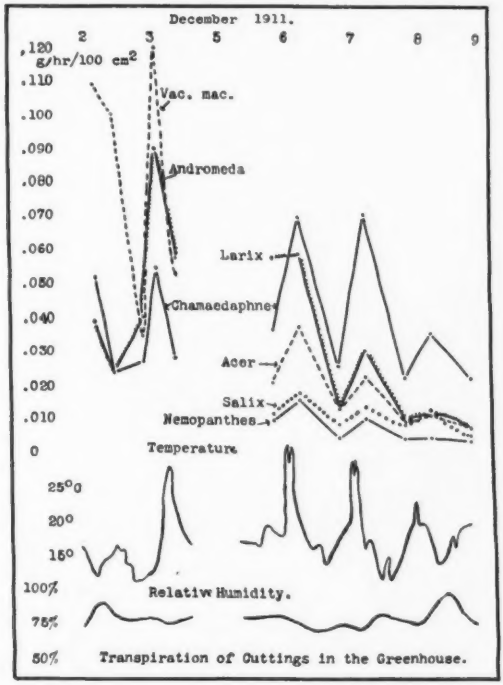


FIG. 3.—Transpiration of cuttings of *Acer rubrum*, *Andromeda glaucophylla*, *Chamaedaphne calyculata*, *Larix laricina*, *Nemopanthes mucronata*, *Salix pedicellaris*, and *Vaccinium macrocarpon* in the greenhouse.

underground and thoroughly protected from exposure, no comparison could be made with the ericads which retain their plant body subject to constant exposure throughout the winter.

The purpose of the winter experimentation, therefore, was to obtain a knowledge of the transpiration of several of the shrubs and trees, and compare that of the leaf-retaining ericads with that of the deciduous shrubs, under winter conditions outdoors and under

laboratory conditions which simulated the severest conditions which could obtain in nature during the winter.

Experimentation was carried on both with potted plants and with cuttings indoors and outdoors. A few of the graphs obtained from the data from these experiments are given in figs. 3–6. Although they have been selected from the general array of data to avoid needless repetition they represent the general conclusions, not merely special cases.

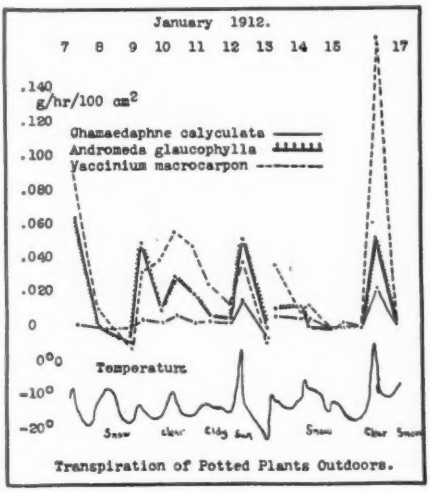


FIG. 4.—Transpiration of potted plants of *Andromeda glaucophylla*, *Chamaedaphne calyculata*, and *Vaccinium macrocarpon* outdoors in winter.

Consideration of these data clearly indicates that the transpiration of these bog plants is very low in winter. Furthermore, with scarcely an exception, the rate of water loss is much greater (2–15 times) in the evergreen ericads than in the leafless shrubs and trees. When the very much more exposed position of the deciduous trees and most of the deciduous shrubs is taken into account, the difference in the rate of transpiration in nature is accentuated. The mere position of the ericads near the ground serves to reduce water loss. This same relation holds among the ericads themselves, namely, that the greater the rate of transpiration under given conditions, the more protected is the position in which that species grows. For example, *Chamaedaphne* transpires at a lower rate than *Andromeda* and *Vaccinium;* and *Chamaedaphne*, because of its higher growth, is more exposed. YAPP (52) has shown that the nearer the ground in a closed association the lower the evaporating power of the air.

These data support the well known facts that transpiration

varies directly with the temperature, inversely with the relative humidity, and is greater in daylight than in darkness. The last may be almost entirely included in the two former, as the absorption of radiant energy during the day would increase the temperature of the leaf were it not used up in augmenting transpiration. For example, on February 27–29, with a temperature constant to within 2° and the relative humidity constant to within 5 per cent, in plants of *Chamaedaphne* which were run in the laboratory with light about 0.1 per cent of sunlight, not at any time being exposed to direct sunlight, the rate of transpiration was very noticeably higher (0.071 gm. and 0.083 gm.) during the diffuse daylight than in the periods of darkness before and after. During the night following, with the temperature constant but the humidity dropping slightly, the transpiration of these same duplicates decreased to 0.046 gm. and 0.060 gm. respectively.

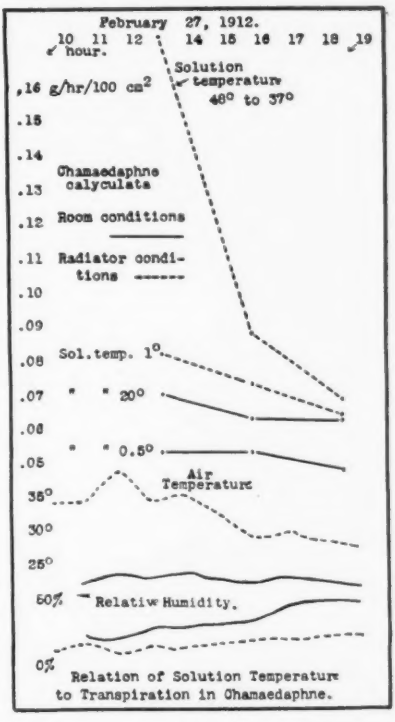


FIG. 5.—The relation of solution temperature to transpiration in *Chamaedaphne calyculata*.

Only rarely does the rate of transpiration exceed 0.01 gm./hr./100 sq. cm. in any of the plants experimented with under winter conditions. With the exception of *Vaccinium macrocarpon*, the rate was more often less than 0.005 gm. than above it. In the deciduous shrubs it was usually below 0.001 gm., and not infrequently was hardly within the power of measurement with

the means employed. The advantage of the deciduous habit in reducing transpiration in the winter is apparent.

Per unit area of leafless twig surface, bog shrubs lose less water than do the bog trees under the conditions of these experiments. Furthermore, bog shrubs are more subject to winter killing in a severe winter in this latitude unless protected by snow. This would seem to indicate that the xerophytism of the bog trees was in general more efficient than that of the bog shrubs under extreme winter conditions, or that their means of water renewal was more efficient.

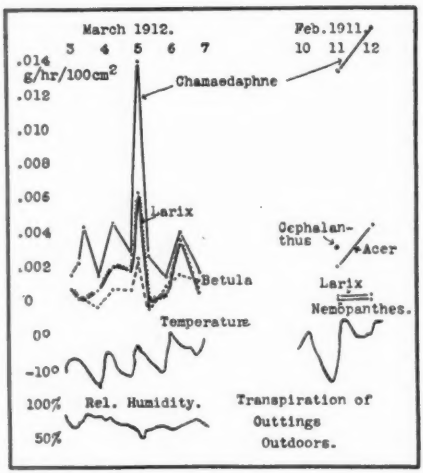


FIG. 6.—Transpiration of cuttings of *Acer rubrum*, *Betula pumila*, *Cephalanthus occidentalis*, *Chamaedaphne calyculata*, *Larix laricina*, and *Nemopanthes mucronata* outdoors in winter.

During stormy weather it is difficult to allow a real exposure to the weather and then be able to measure transpiration by weighing, but repeatedly, even when preparations were shielded from the rain or snow, measurement showed an increase in weight beyond that of a control. This was noticed particularly in *Chamaedaphne* and *Andromeda*, whose leaves were covered beneath with scales and hairs, respectively. This absorption helps replenish the saturation deficit of the leaves, and so in a measure alleviates the demands upon the root system at a time of year when absorption is at best difficult. It is not certain, however, whether this water absorbed from the air ever actually penetrates to the mesophyll.

*Influence of solution temperature upon transpiration*

To determine the influence of the temperature of the solution from which the cutting was taking water upon the rate of tran-

spiration, a number of experiments were made in the laboratory, the results of which are summarized in table I.

TABLE I

SUMMARIZING THE EFFECT OF LOWERING THE TEMPERATURE OF THE SOLUTION UNDER THE SAME EVAPORATING POWER OF THE AIR. THE TRANSPIRATION IS EXPRESSED IN GRAMS PER HOUR PER 100 SQ. CM., AND THE NUMBER OF MEASUREMENTS UPON WHICH THE AVERAGE IS BASED IS GIVEN IN PARENTHESES

*Under room conditions* (temp. 18–25° C.; rel. hum. 30–40 per cent)

| | Solution at room temperature | Solution at freezing temperature |
|---|---|---|
| Chamaedaphne (Chamaedaphne association)..... | 0.062 (13) | 0.054 (13) |
| Chamaedaphne (Carex association)..... | 0.080 ( 2) | 0.068 ( 4) |
| Chamaedaphne (Larix association)..... | 0.042 ( 2) | 0.042 ( 3) |
| Larix laricina (Larix association)..... | 0.022 ( 4) | 0.028 ( 4) |

*Under radiator conditions* (temp. 38–50° C.; rel. hum. 4–20 per cent)

| | Solution at radiator temperature | Solution at freezing temperature |
|---|---|---|
| Chamaedaphne (Chamaedaphne association)..... | 0.141 (12) | 0.067 (11) |

In the course of these experiments cuttings were made during the cold snowy weather of February and March 1912, and subjected to different conditions in the laboratory. The laboratory conditions of temperature and relative humidity are more extreme than these plants are ever naturally subjected to in winter.

To determine the influence of a cold source of water supply, twigs were set up in water in bottles, set in a snow mixture, which kept the temperature of the solution at or near freezing. Other twigs set up in the room gave data for comparison. The general results show a lower rate of transpiration from the colder solution. The effect, however, is not so pronounced as might be expected. The control was much less perfect in leafless twigs than in leafy ones. Leafless twigs have occasionally shown a higher rate of transpiration from the colder solution. This is probably due to the stimulation which is the first effect of application of cold (BOSE 2). Such a higher rate, however, is only temporary, although it has continued for 10 hours in some of the leafless twigs experimented upon. Warming the solution was uniformly accompanied by a rise in the rate of transpiration. A lowering of the rate of

transpiration from a colder solution would be caused by the extra amount of energy required to warm the colder water up more degrees of temperature. In nature these bog plants are subjected to these extremes of temperature maintained in the laboratory, and this range is well within their physiological ability.

These experiments were repeated under the very severe conditions obtaining over a steam radiator. As the temperature of the solution became hot, transpiration proceeded at a very rapid rate, until the tissue was killed at a temperature of 42–46° C., after which the transpiration fell to a low amount without a corresponding drop in the evaporating power of the air. The graph obtained by plotting the data may be called the death curve. It is similar to those obtained by BOSE (2) in experimental work on death in plant tissues. After the marked drop, the rate of transpiration increases and then fluctuates with the evaporating power of the air, but does not exhibit the decided increase which the access of sunlight causes in living twigs.

Other twigs maintained in cold water under radiator conditions exhibited a higher rate of transpiration than those in the room. The average rate of transpiration under these conditions was very little in excess of that of twigs in the room with solution at room temperature. This clearly shows the retarding influence of cold soil water. It also shows the ability which these plants possess of withstanding a much severer aerial condition of high temperature and low relative humidity than they are ever subjected to in nature.

### *Rate of conduction of a 0.5 per cent aqueous solution of lithium nitrate*

Table II summarizes the winter experimentation upon the rate of conduction in peat bog plants.

The results obtained from these experiments were subject to considerable variation, but in general the rate of conduction was greater from warmer solutions. Plants from habitats where the soil temperature is naturally lower seem to have a greater ability to conduct water from a colder solution. Transpiration takes place faster from a warmer solution. An increase of rate of conduction from a warmer solution is just what is to be expected to supply the greater demand for water which increased transpiration entails.

TABLE II

*Under room conditions* (temp. 18–24° C.; rel. hum. 27–45 per cent)

| | Solution at warmer temperature (20°) | Solution at colder temperature (0°) |
|---|---|---|
| | cm./hr. | cm./hr. |
| Chamaedaphne (Chamaedaphne association) | 3.1 (29) | 2.5 (33) |
| Chamaedaphne (Carex association) | 3.2 (13) | 3.1 (12) |
| Chamaedaphne (Larix association) | 3.5 (14) | 4.3 (12) |
| Acer rubrum | 0.8 ( 3) | 0.6 ( 4) |
| Spiraea salicifolia | 2.1 ( 2) | 1.3 ( 2) |
| Betula pumila | 2.4 ( 3) | 2.0 ( 3) |
| Larix laricina | 3.0 (17) | 2.7 (19) |

*Under conditions found over steam radiator*

| | Solution at warmer temperature (40°) | Solution at colder temperature (0°) |
|---|---|---|
| Chamaedaphne calyculata | 3.0 ( 9) | 2.8 ( 9) |
| Larix laricina | 5.1 ( 4) | ..... |

Experiments that were run outdoors showed a rate of conduction less than 1 cm./hr. Conduction continued to take place even after the solution was frozen.

## *Relationship between rates of conduction and transpiration in evergreen ericads*

Tables III and IV summarize the experimental results.

TABLE III

EXPERIMENTS OF APRIL 4–6, 1912

*Rate of conduction in centimeters per hour*

| | Chamaedaphne calyculata | Andromeda glaucophylla | Vaccinium macrocarpon |
|---|---|---|---|
| April 4 | 5.90 (7) | 7.5 (13) | ..... |
| April 5 | 4.90 (9) | ..... | 8.3 (12) |
| April 6 | ..... | 11.1 (12) | 16.3 (11) |
| Average | 5.34 | 8.43 | 12.11 |
| Ratio | 1.00 | 1.58 | 2.27 |

*Rate of transpiration in grams per hour per 100 sq. cm. of leaf surface*

| | Chamaedaphne calyculata | Andromeda glaucophylla | Vaccinium macrocarpon |
|---|---|---|---|
| April 4 | 0.140 (2) | 0.230 (2) | ..... |
| April 5 | 0.101 (2) | ..... | 0.301 (2) |
| April 6 | ..... | 0.290 (2) | 0.527 (2) |
| Average | 0.121 | 0.260 | 0.414 |
| Ratio | 1.00 | 2.15 | 3.42 |

TABLE IV

CHAMAEDAPHNE CALYCULATA

| | February 27 | March 1 | March 3 | March 5 |
|---|---|---|---|---|
| Laboratory (room temp.)... | $\frac{0.067}{2.4}=0.028$ | $\frac{0.051}{1.8}=0.028$ | $\frac{0.043}{2.8}=0.015$ | ..... |
| Laboratory (sol. freezing) .. | $\frac{0.052}{1.9}=0.027$ | $\frac{0.052}{2.3}=0.023$ | ..... | $\frac{0.060}{2.5}=0.024$ |
| Radiator (rad. temp.)..... | $\frac{0.111}{2.9}=0.038$ | $\frac{0.199}{2.0}=0.099$ | ..... | ..... |
| Radiator (sol. freezing)..... | $\frac{0.073}{2.5}=0.029$ | $\frac{0.61}{2.6}=0.023$ | ..... | ..... |
| Outdoors (sol. frozen)..... | $\frac{0.010}{0.5}=0.020$ | $\frac{0.007}{0.8}=0.009$ | $\frac{0.003}{0.7}=0.004$ | ..... |

To obtain the factor in table IV, the average of the values of all specimens under the same conditions during a day was used as a unit. The rate of transpiration was divided by the rate of conduction. The resulting factor expresses the number of grams of transpiration per unit area, which is equivalent to 1 cm. of conduction. With the solution at a lower temperature in one of two simultaneous sets, but the evaporating power of the air the same, it takes smaller amount of transpiration to account for 1 cm. of conduction.

Utilizing the high temperature of the steam radiator in the laboratory, it was found that a much greater (2–4 times) amount of water loss occurred in proportion to the amount of conduction in plants kept in solutions at the radiator temperature than near freezing. The temperature of the radiator exceeded the death point of the plants and was far above any temperature that their roots are ever subjected to in nature.

Plants maintained in solutions near the freezing point in the extreme evaporating condition of the air above the radiator, a condition which is more severe, with respect both to summer heat and to summer soil water temperatures, than any ever experienced by *Chamaedaphne* in its natural habitat, exhibited a higher transpiration and a higher rate of conduction than plants kept in the room at both room and freezing temperatures. This was in spite of the cold solution. A comparison of the transpiration-conduction factor of plants in freezing solutions in the room and over the radiator shows scarcely any difference.

With cuttings experimented upon outdoors it took even less transpiration for a given amount of conduction.

The logical conclusion substantiated by these data is that the ability of plants of *Chamaedaphne* to conduct water is at all times, when above $-15°$ C. (the lowest temperature at which experiments were performed), greater than the necessity, and that xerophytic modification in any part of the plant is not a result of the lack of ability to conduct water.

The occurrence of temperatures of $-15°$ is frequent in the natural range of these plants and no injuries are apparent. At a temperature lower than $-25°$, such as occurred during February 1912, the drying up of the exposed leaves, twigs, and flower buds of *Chamaedaphne* is first hand evidence that at such a low temperature the conduction in the upper part of the plant is not sufficient to supply the transpiration demands.

### *Relation of winter to the xerophily of peat bog ericads*

Among peat bog plants, ericads, with but few exceptions, retain their leaves during the winter, when most other plants are leafless. This is usually considered a matter of heredity. The presence of leaves very materially increases the evaporating surface of the plant and thereby enhances the demand for water from the habitat.

That the different ericads experimented with differed among themselves in their transpiring ability with their degree of protection from excessive evaporation during the winter, and that *Chamaedaphne*, the least protected, transpires considerably more than the leafless shrubs and trees, indicates both the effectiveness of the deciduous habit as a xerophytic adaptation and the necessity of there being other xerophytic modifications in the case of the ericads. Added to this, the difficulty of absorbing water is apparent, due, if for no other reason, to the colder temperature of the bog soil in winter, and the necessity of xerophytic modification. The presence of so many of the usual xerophytic modifications in these peat bog ericads is noteworthy. The thick cuticle, dense palisade layer, more or less sunken stomates, hairs, scales, bloom, and waxy coverings, resin, and the upright position of the leaves are all indications

of this xerophily, this necessity of keeping the transpiration within the limits of absorption and conduction.

From the experimental work it was evident that, supplementing any water vapor absorbed by the leaves, the rates of absorption and conduction in the evergreen ericads are more than ample to furnish sufficient water for transpiration during warm spells in winter and for continued periods of cold temperature down to at least $-15°$. When, however, continued temperatures lower than $-20°$ prevail in this region, the rate of conduction is not sufficient to prevent drying and death of the exposed parts of the plant. On this point the winter of 1911–1912 gave remarkable evidence. Consequently, a high degree of xeromorphism is demanded to compensate for a lack of a decrease of leaf surface during the winter.

## EXPERIMENTATION DURING THE SUMMER

### *Transpiration*

The water loss of plants is greatest in amount during the summer or growing season. The greater evaporating power of the air, the greater exposure of surface of most plants, the greater availability of water, and the use of water in photosynthesis all express the direct opposite of the conditions in winter and show the greater need for water in the summer. The results of a few typical experiments have been plotted in figs. 7–9.

During the first week of July 1912, the transpiration of three series of potted plants was determined at frequent intervals (fig. 10). As this week was one of extremely hot, dry weather, it will serve to indicate the limits of transpiration to which these plants may be subjected in summer. At the beginning of each series of potted plants a number of cuttings were also run to enable a comparison between cuttings and potted plants of the same species.

With the potted plants it is noteworthy that, with but a single exception, the period of maximum transpiration was reached at the time of maximum evaporating power of the air, a little before the middle of the afternoon. The graphs of evaporating power of the air and those of transpiration of potted plants are very similar. Per unit area, however, between three and five times as much

water is evaporated from a free water surface as from leaf surface on hot days.

With cuttings somewhat different results were obtained. With occasional exceptions, especially on less extreme days, the maximum daily transpiration was reached before noon, 2–4 hours before that

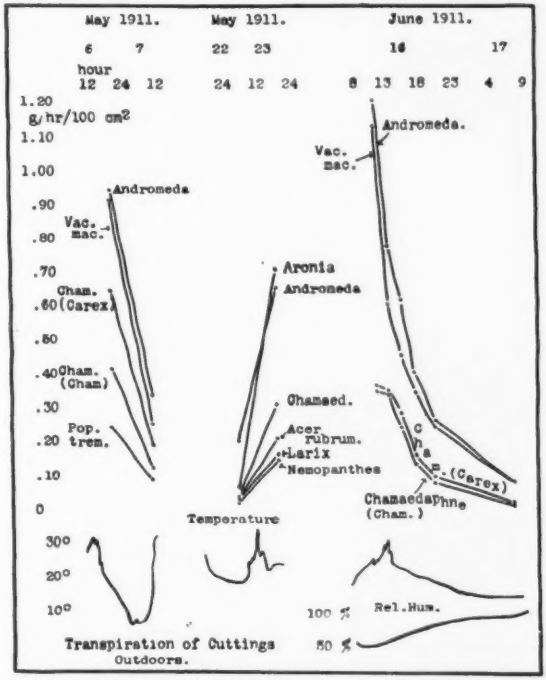


FIG. 7.—Transpiration of cuttings of *Acer rubrum*, *Andromeda glaucophylla*, *Aronia melanocarpa*, *Chamaedaphne calyculata* (from both the *Carex* and the *Chamaedaphne* associations), *Larix laricina*, *Nemopanthes mucronata*, *Populus tremuloides*, and *Vaccinium macrocarpon* outdoors.

of the maximum evaporating power of the air. Exceptions were more general in shrubs and especially in *Chamaedaphne*. In this species, whose rate of transpiration is low at all times, even in cuttings, the maximum transpiration accorded closely with the maximum evaporating power of the air.

A comparison of the results of potted plants and cuttings of the same species, although exhibiting some variation, shows that the rate of transpiration is greater in the potted plant. The difference is usually greatest at the time of maximum evaporating power of the air. Under less extreme conditions of evaporation, the

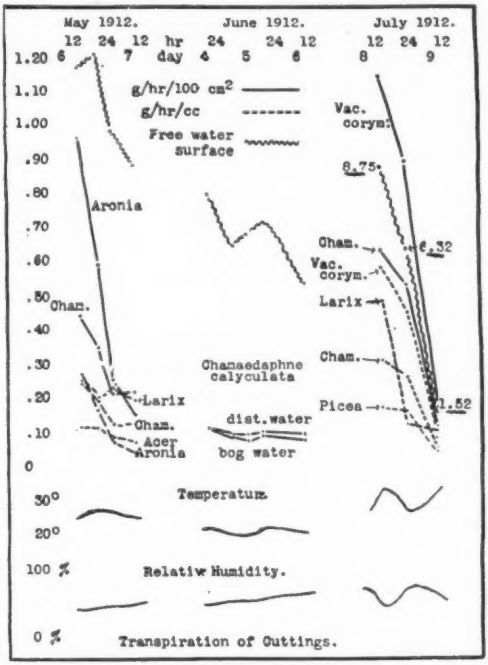


FIG. 8.—Transpiration of cuttings of *Acer rubrum*, *Aronia melanocarpa*, *Chamaedaphne calyculata*, *Larix laricina*, *Picea mariana*, and *Vaccinium corymbosum* outdoors, and of cuttings of *Chamaedaphne calyculata* from bog and distilled water in the laboratory.

graphs of transpiration of cuttings accord more closely with those of potted plants. From this it follows that one can obtain a knowledge of relative values under moderate conditions with cuttings, but experimentation under extreme conditions of evaporating power of the air with cuttings is unsatisfactory.

From an inspection of all of the summer data, it is seen that

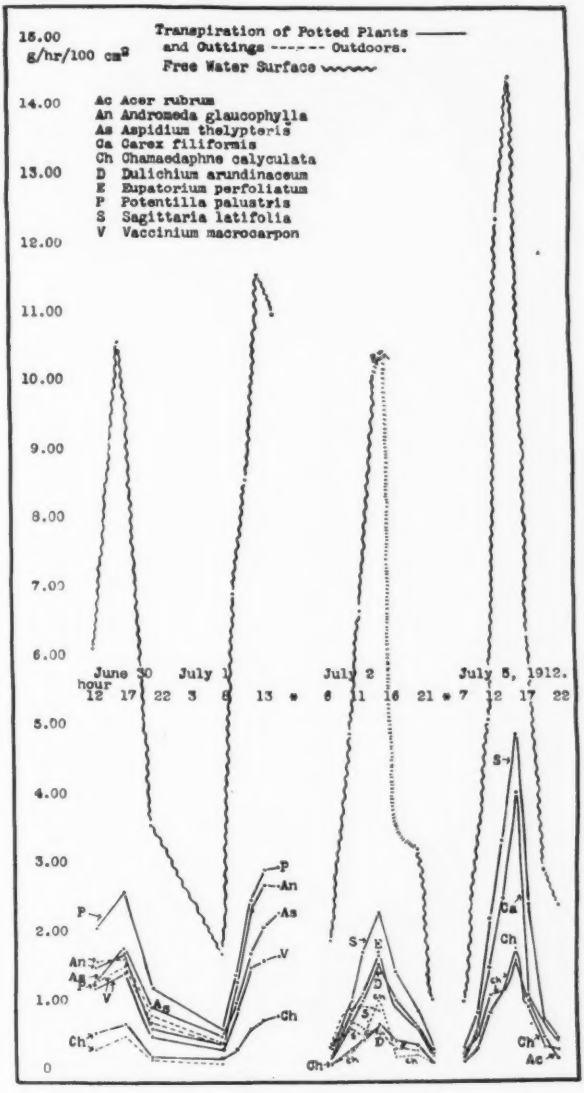


FIG. 9.—Transpiration of certain potted plants and cuttings during a severe hot wave.

among the shrubs, *Chamaedaphne* exhibits the lowest rate of transpiration. Herbaceous plants in general show a higher rate of transpiration than shrubs, while trees usually have a lower rate. Among plants that may be exposed to winter conditions, evergreen trees and shrubs transpire at a lower rate than deciduous trees and shrubs.

FIG. 10.—A view of potted plants and cuttings in the course of a transpiration experiment during a hot wave; July 2, 1912.

### *Rate of conduction*

For use in these experiments cuttings were made in the evening after the stomates had closed, and kept under laboratory conditions over night, where the sun could strike them in the early morning, after which the experimentation was carried on as usual. Consequently, the results are not maximum rates of conduction, but relative rates of different species under similar conditions. In the summary of this work (table V), each set of

experiments is grouped together, and each figure represents at least two determinations.

TABLE V

SUMMARY OF SUMMER EXPERIMENTATION (1912) ON RATE OF CONDUCTION OF PEAT BOG PLANTS; RESULTS IN CENTIMETERS PER HOUR

| | July 6 | July 6 | July 8 | July 9 |
|---|---|---|---|---|
| Temperature | 31° | 33° | 25° | 26° |
| Relative humidity | 59 per cent | 48 per cent | 69 per cent | 69 per cent |
| Chamaedaphne calyculata | 12 | 20 | 13 | 22 |
| Andromeda glaucophylla | 21 | ..... | ..... | ..... |
| Vaccinium macrocarpon | 23 | ..... | ..... | ..... |
| Vaccinium corymbosum | ..... | ..... | 60 | ..... |
| Larix laricina | (31*) | ..... | 47 | ..... |
| Picea mariana | ..... | ..... | 26 | ..... |
| Acer rubrum | ..... | 70 | ..... | ..... |
| Cephalanthus occidentalis | (27*) | (140*) | ..... | (170*) |
| Aronia melanocarpa | (51*) | ..... | ..... | 108 |
| Salix pedicellaris | (35*) | ..... | ..... | ..... |
| Nemopanthes mucronata | (40*) | ..... | ..... | ..... |
| Spiraea salicifolia | ..... | 48 | ..... | ..... |
| Sambucus canadensis | ..... | ..... | ..... | 180 |
| Aspidium thelypteris | (30*) | ..... | ..... | 66 |
| Asclepias incarnata | ..... | ..... | ..... | 210 |
| Eupatorium perfoliatum (wilted) | ..... | ..... | ..... | 84 |
| Sagittaria latifolia (wilted) | ..... | ..... | ..... | 49 |
| Hypericum virginicum | ..... | ..... | ..... | 126 |
| Potentilla palustris | ..... | ..... | ..... | 86 |
| Typha latifolia | ..... | ..... | ..... | 114 |
| Carex filiformis | ..... | ..... | ..... | 90 |
| Sarracenia purpurea | ..... | ..... | ..... | 102 |

*Figures followed by an asterisk and inclosed in parentheses indicate that the lithium nitrate had reached the tip of the stem within the time of experimentation.

From an inspection of the data of table V it is readily seen that the evergreen ericads experimented with are all characterized by a low rate of conduction, decidedly low in comparison with that of both herbaceous plants and other shrubs. The rate of conduction was markedly lower in the evergreen bog trees than it was in the deciduous bog trees. Southern swamp shrubs showed a higher rate of conduction than northern bog ones.

*Relation of summer to the xerophily of peat bog ericads*

During the summer all the peat bog plants are actively carrying on the work of the growing season and have their greatest need of water. Especially at the beginning of the growing season the soil

water is cold, more so in the tree and shrub associations than in the herbaceous ones. This necessitates conservation of water and consequently xerophytic adaptation. Leaves that are produced in a time of drought (June 1912 at Mud Lake) are more xerophytic, in that they remain upright, than those produced under normal conditions (May 1912 at First Sister Lake, fig. 11). Neither observations in the extreme summer drought of 1911 in Cheboygan

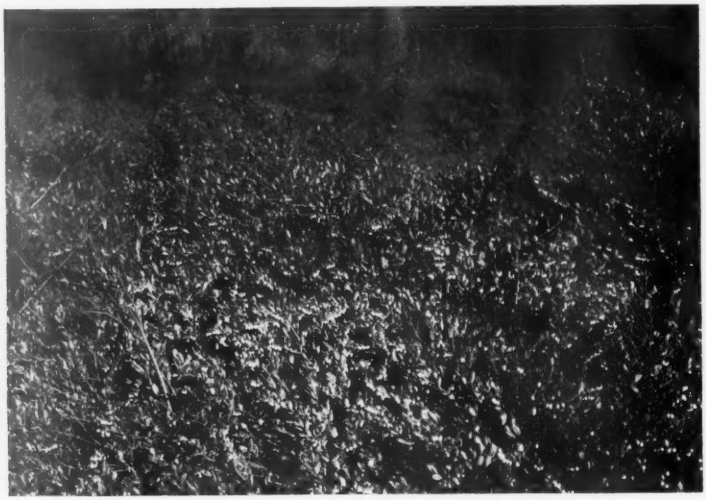

FIG. 11.—*Chamaedaphne calyculata* in bloom at First Sister Lake; May 6, 1912

County, Michigan, nor experimentation under severer conditions than obtain in nature made it apparent that such a degree of xeromorphism as exhibited by *Chamaedaphne* was necessitated by the summer conditions alone.

ABSORPTION OF WATER VAPOR FROM A SATURATED ATMOSPHERE

From the increases in weight repeatedly obtained while experimenting with potted plants and cuttings of ericads during times of high relative humidity, it seemed necessary to assume the ability to absorb a measurable amount of water from the water vapor in the air, at a time when the evaporating power of the air was low. During June and July 1912, some measurements were made of the

changes in weight which took place when leafy twigs were subjected to dry air and moist air under bell jars. A twig of *Chamaedaphne* which lost water at the rate of 0.026 gm. per hour per 100 sq. cm. of leaf surface in dry air, while under a bell jar with a dish of water gained at the rate of 0.010 gm. Twigs of *Picea mariana* and *Larix laricina* gained in weight in moist air at the rate of 0.00027 and 0.000034; while those in dry air lost at the rate of 0.0061 and 0.0111 gm. per hour per cc. of volume, respectively.

These experiments demonstrate the ability of the living leaves of the three evergreen ericads and two conifers used to absorb water vapor in measurable quantities from saturated air. The amount so absorbed has increased the weight of a leafy twig by 6 per cent in 4.5 hours. When one considers the low position of bogs and the presence of heavy dew practically every night, no inconsiderable quantity of water may be accumulated in this way and furnish a part of the water for evaporation during the day and so lessen the demand upon the roots.

Dead leaves of *Chamaedaphne* absorb considerably more (3–6 times) than living ones. The scales which are present in abundance on the lower sides of the leaves no doubt take an active part in this absorption. Their structure is very similar to those of certain epiphytic orchids (*Vriesia*) which have been shown to be water-absorbing.

This function may serve to explain the excessive abundance of scales produced on the leaves developing in the excessively dry month of June 1912 at Mud Lake, in opposition to the smaller number on the leaves of the plants at First Sister Lake which developed in May before drought conditions set in.

The lessening of the drain upon the root system, when absorption is at best difficult, is no doubt greatly assisted by vapor absorption by the leaves. The prevalence of dew and frost in the bogs even when absent on the uplands indicates the greater humidity of the bog conditions. The actual presence of snow in contact with the leaves greatly aids in two ways: in checking water loss, and in constantly furnishing water vapor which may be absorbed.

The effect of absence of snow protection in time of cold, dry weather was admirably shown in the vicinity of Ann Arbor during

the winter of 1911–1912 (GATES 19). The bushes of *Chamaedaphne* were uniformly killed down to the snow level. This might have been due to the cold itself, but more likely was the result of excessive drying beyond the limits of the plants to recuperate. The difference in temperature at the snow level and 3 cm. below it was scarcely noticeable, upon the occasions when it was determined, during the moderately cold weather (−15 to 20°), yet above the snow line the plants died. At low temperatures the absolute amount of water vapor that can be in the air is very low, but below the line of snow the air is saturated. This argues for death by drying rather than by freezing, for the leaves below the snow line have more chance to keep within the drying limits of their protoplasm.

### EXPERIMENTATION UPON THE CONDITION OF THE STOMATES

As has already been outlined, the data obtained upon this question are based upon the time it took a penetrating liquid (xylol) to render the leaf translucent. Different species varied in the readiness with which they became translucent, but a little experimentation soon sufficed to disclose a "normal" rate upon which to base deductions.

During the entire course of the experiments upon the several species of bog plants, the relative time of penetration indicated that the stomates were always open during the time that the full sun shone upon them. For many plants the diffuse light of early morning was sufficient to open the stomates partially, but it did not always appear that they were wide open until the full sun shone upon them. In other species, as *Nymphaea advena*, the stomates did not open until the direct sun reached the leaves. So far as was observed, clouds did not cause the closing of the stomates on hot, dry days, but on cloudy days the stomates closed earlier in the afternoon than on sunny days.

Just after the time of maximum transpiration and maximum evaporating power of the air, the stomates begin to close. The rate of transpiration drops very quickly, but stomates close slowly, and in most of the species are not completely closed until after dark. In a few species, as *Salix pedicellaris*, there was no evidence that the stomates ever closed.

Plants that were collected in the morning always had their stomates wide open at the time of collection. Before the laboratory was reached, however, the plants were frequently wilted, and investigation always showed the stomates closed. If not too severely wilted, the plants survived within an hour after cutting under water under laboratory conditions of diffuse light. When revived, the stomates opened partially in the diffuse light, but opened wide when exposed to sunlight.

Collections in the early afternoon of summer days were abandoned because of the difficulty of getting the material into the laboratory without extreme wilting. At this time of day the stomates were always wide open in the field, but closed upon wilting.

Collections made just before dark showed different results for different plants. The sun no longer shone upon the leaves, although it was by no means dark. Most of the leaves were very slowly penetrated by the xylol, which indicated that the stomates were but partially open. In some species (*Nymphaea*) the stomates were closed tight and no penetration would take place even in two minutes.

Some experimentation was performed on material kept in the laboratory with the following results. Wilted plants (*Aspidium thelypteris*, *Dulichium arundinaceum*, *Menyanthes trifoliata*, *Nymphaea advena*, *Potentilla palustris*, *Eupatorium perfoliatum*, *Acer rubrum*, *Spiraea salicifolia*, and *Vaccinium macrocarpon*) invariably had their stomates closed, as the length of time it took the xylol to penetrate the leaves testified. One single exception was found to be *Salix pedicellaris*, in which there was a scarcely perceptible increase in the time of penetration of wilted specimens as compared with plants in the field. If wilting was allowed to continue until the leaves became dry, the almost instantaneous penetration gave proof of the open condition of the stomates in the material used (*Spiraea salicifolia*, *Nemopanthes mucronata*, *Aronia melanocarpa*, *Salix pedicellaris*, *Chamaedaphne calyculata*, *Potentilla palustris*, *Ilex verticillata*, *Cephalanthus occidentalis*, *Menyanthes trifoliata*, *Carex filiformis*, and *Larix laricina*). Those of *Gaylussacia baccata* were only partially open.

Leaves of *Salix pedicellaris*, *Andromeda glaucophylla*, *Chamae-*

*daphne calyculata*, *Aronia melanocarpa*, *Potentilla palustris*, *Acer rubrum*, and *Cicuta maculata* were kept in the laboratory window submerged in water for a couple of days. Xylol determinations disclosed the fact that the stomates were open both day and night. Only in *Chamaedaphne* was there a perceptibly longer penetration time after dark than in the day.

This method of experimentation upon living plants in the field, which gives results so very quickly, leads to the view that stomatal movements and variations of the rate of transpiration are to a high degree independent of each other. Light is the fundamental cause for change in the first and the evaporating power of the air in the latter. The fact that the maximum evaporating power of the air occurs during the period of light does not necessarily mean that the stomates regulate transpiration. It has been shown that transpiration can change independently of stomatal movement (LLOYD 32). BROWN and ESCOMBE (53) have shown that the outward diffusion of water vapor observed is not as great as the capacity of the stomates will permit. The check in transpiration during the day comes with decline of the evaporating power of the air, and the stomates begin to close *soon after*, with the diminishing light intensity. There is no doubt that closed stomates retard water loss, and that stomatal movements and transpiration changes often occur in conjunction; but that is not proof that one is the cause and the other its effect.

## Conclusions

Water loss was demonstrated in every plant experimented with during the winter as well as during the summer. During the low temperatures of winter this water loss was very low, although it continued to take place even when the water was frozen around the stem. Either this water loss was a permanent drain on the water content of the stem or, as the experiments on conduction showed, there was a certain amount of renewal from the external frozen solution. Whether a plant can obtain water directly from an ice surface is not known, but it has been shown by KOSAROFF (25) and others that plant roots can absorb water vapor when the temperature is below freezing. The amounts, though small, are

usually sufficient to satisfy the needs of the plants. In nature, the ground, although often frozen to a slight depth, is usually protected by a snow cover during the winter (fig. 12). The spaces between the snow particles are saturated with water vapor. This produces a blanket effect, greatly reducing the temperature changes beneath the snow level and tending to keep the temperature nearer the freezing point in times of cold. Evaporation from the snow also furnishes an available form of water in winter.

FIG. 12.—A view in the Mud Lake bog when *Chamaedaphne* was buried under 80 cm. of snow; March 26, 1912.

The evergreen ericads are distinctly grouped apart from the deciduous plants. The transpiration of the former in winter was 2–15 times as great as in the latter. The evergreen habit, even with a noteworthy accumulation of recognized xerophytic structures, was not as efficient a protection as the deciduous habit. This the universal killing of the plants of *Chamaedaphne* above the snow line in the extremely severe winter of 1911–1912 demonstrated, while other evergreen plants, not so intensely xeromorphic, but entirely covered with snow, were unharmed.

During the summer the water loss was much greater than in the

winter, especially, to be sure, in plants of the deciduous habit. Transpiration bore a rather definite relation to the evaporating power of the air. This relation varied both with species and with individuals. Its numerical value was from one-third to one-twelfth that of the air. It was obvious how efficient the xeromorphic structure of the evergreen trees and shrubs is in lowering the rate of transpiration during the summer. During the winter the absolute value of transpiration of *Chamaedaphne* varied up to 0.02 gm. per hour per 100 sq. cm. of leaf surface as a maximum under outdoor conditions, and 0.07 gm. under indoor conditions, and did not exceed 0.30 gm. under conditions above a hot radiator, which were severer than ever experienced in nature. Transpiration from distilled water was 1.09 times that from bog water. During the summer the normal rate at night is less than 0.10 gm., while in the daytime it approaches 0.80 gm. Only on extremely hot days with low relative humidity did the rate exceed 1.00 gm. The maximum recorded was 1.70 gm./hr./100 sq. cm. This means that the average summer rate of transpiration in *Chamaedaphne calyculata* is 25–30 times greater than the maximum winter rate, while the maximum summer rate was over 80 times that of the maximum winter rate. For deciduous plants the difference is very much greater.

The highest rates of water loss were in the more hydrophytic plants, *Nymphaea advena*, *Sagittaria latifolia*, and *Carex filiformis*, which agrees with the experimental data obtained by Otis (**36**) by a different method. In a potted plant of *Sagittaria* the maximum rate obtained during the investigation occurred on the afternoon of July 5, 1912 (4.82 gm./hr./100 sq. cm.).

As has been stated above and is here briefly repeated, during the winter the transpiration and rate of conduction of water are much higher in the evergreen plants (*Chamaedaphne calyculata*, *Andromeda glaucophylla*, *Vaccinium macrocarpon*, and *Picea mariana*) than in the deciduous ones. In the summer the rate of transpiration and conduction in the herbaceous plants (*Nymphaea advena*, *Sagittaria latifolia*, *Eupatorium perfoliatum*, *Asclepias incarnata*, *Dulichium arundinaceum*, *Aspidium thelypteris*, *Hypericum virginicum*, *Carex filiformis*, *Cicuta maculata*, and *Potentilla*

*palustris*) and in the deciduous woody plants (*Aronia melanocarpa*, *Nemopanthes mucronata*, *Salix pedicellaris*, *Cephalanthus occidentalis*, *Spiraea salicifolia*, *Gaylussacia baccata*, *Vaccinium corymbosum*, and *Larix laricina*) is much higher than in the evergreen shrubs and trees. The more xeromorphic the structure of the leaves the lower is the transpiration, and the more exposed to winter conditions the more xerophytic is the structure. This argues well for the winter conditions as being the fundamental causes necessitating xerophily in the evergreen ericads. The difficulty is in not being able to obtain water sufficiently fast or in sufficient quantities to supply the demands of transpiration. This is very much accentuated in the winter, mainly on account of low temperatures.

Although this xerophytic structure also reduces the water demands of these plants during the summer, there does not appear to be in summer any need of so thorough a xerophily, for neither the very extreme droughts and hot spells of the summers of 1911 and 1912, nor the extreme evaporating conditions of the laboratory appreciably injured the many plants of *Chamaedaphne* observed or experimented upon. On the other hand, the continued severe dry cold during the winter of 1911–1912 killed thousands of plants of *Chamaedaphne* down to the snow line, below which they were efficiently protected, whereas during the average winter of 1910–1911, with but very little snow on the ground during the coldest weather (barely below $-17°$ C.), all of the bushes of *Chamaedaphne* remained alive clear to the top.

As these ericads are of distinctly northern phytogeographic affinities (HARSHBERGER **21, 22,** and TRANSEAU **48**), and consequently are less subject to the extremes of summer conditions which obtain near Ann Arbor, almost at the southern limit of their range, and are more subject to severe winter conditions in nature farther north, and as the evergreen habit is a hereditary character, the xerophytism is real and has been brought about and is necessitated primarily by the winter conditions which these plants endure.

## Summary

1. The determination of the rate of transpiration per unit area of leaf surface by weighing is a satisfactory approach to a knowledge

of the demands of plants for water, one of the most essential features of their environment. This unit was taken as grams per hour per 100 sq. cm. of leaf surface.

2. While the use of rooted plants is most desirable, the results obtained through measuring the transpiration of cuttings, the stomates of which are closed at the time of collecting and during the period of adjustment to the apparatus, approach closely those of potted plants, except under extreme conditions of evaporating power of the air. Experiments run in duplicate are a check upon the behavior of individual specimens.

3. Cuttings of hydrophytic plants wilt in a very short time in spite of every precaution, and experiments of any duration cannot be made upon them. Cuttings of other herbaceous plants, if properly cared for, may be used for at least 24 hours, and cuttings of shrubs and trees may be used for a still longer time.

4. From the data obtained in this investigation, transpiration varies with the evaporating power of the air, that is, increases directly with increase of temperature, decrease of relative humidity, increase of air movement, and, other factors being equal, is greater in daylight than in darkness. The last factor, however, is due to the tendency toward increased internal temperature with absorption of radiant energy.

5. The transpiration of all plants experimented with was very low in winter, yet was demonstrable during the daytime of clear days, even at the lowest temperatures ($-29°$). Very frequently no transpiration could be detected during the night. The decided gain in weight which frequently occurred is to be attributed to the power possessed by the leaves of these evergreen ericads of absorbing water from a humid atmosphere. A gain always occurred on frosty nights and at times of high relative humidity. Both fresh and dried twigs of the evergreen ericads and of the conifers exhibited an ability to absorb water vapor from humid air. Such absorption is much greater in quantity per unit volume of leafy twig in the evergreen conifer *Picea mariana* than in the deciduous conifer *Larix laricina*, and in the scale-covered *Chamaedaphne* than in the other evergreen ericads. As such absorption not infrequently is three or four times as great as the transpiration

during a very cold winter day, it is obvious how great is the advantage of this ability to lessen the demands upon the root and conducting systems at a time when the ground is frozen solid.

6. The transpiration per unit surface of the evergreen shrubs was very decidedly greater (4–10–30 and more times) than that of the deciduous shrubs during the winter under both outdoor conditions and indoor conditions which simulated warm spells in winter.

7. Among the evergreen ericads the relative rates of transpiration varied in the inverse order of their exposure and of their xerophytic structure. *Chamaedaphne*, which is the most exposed and the most xerophytic, has the lowest rates of transpiration and conduction.

8. During an average southern Michigan winter, protection by snow is not essential to the preservation of *Chamaedaphne*. In an extremely severe winter (1911–1912) absence of snow protection results in the killing of parts not so protected.

9. During the coldest weather (−29°) it did not appear that twigs or leaves of *Chamaedaphne* were frozen, as they were either perfectly pliable to handling or cracked as dry leaves.

10. Experimentation upon the rate of conduction by the lithium nitrate method showed a relatively higher rate at first, following the shock of cutting. Variations in external factors could not be arranged for a given plant because the shortness of the stem necessitated a short time of experimentation, and in order to obtain the data the stem had to be destroyed.

11. The rate of conduction was faster from warmer solutions, but was never zero when the solution was frozen. As the transpiration varied similarly, it seems obvious that the transpiration exercises a general regulatory function on the rate of conduction, similar to that which it exercises upon water absorption. The regulation is not exact, for in incipiently dry plants the rate of absorption and conduction is relatively greater than the rate of transpiration, while in turgid plants transpiration may be greater than absorption or conduction.

12. While the rate of conduction was relatively higher in the evergreen ericads than in other shrubs during the winter, leafless

twigs of *Larix* exhibited virtually as high a rate of conduction, especially from colder solutions.

13. From the experimentation it appears that the rate of conduction is ample to the needs of these plants for temperatures above $-15°$ (and probably above $-20°$).

14. The transpiration of all plants experimented with in summer was very decidedly greater than in winter. This was most marked in deciduous species and least so in evergreen species.

15. Under summer conditions, hydrophytes exhibited the highest transpiration per unit area of leaf surface. In general, herbaceous plants transpired at a higher rate than shrubs. The more hydrophytic swamp shrubs transpired at a higher rate than the typical bog shrubs. The evergreen shrubs transpired at a very distinctly lower rate than the deciduous ones. Among the bog trees the lowest rates occurred in the evergreen species. The rate of the deciduous conifer *Larix laricina* was noticeably higher than that of the deciduous broad leaf tree *Acer rubrum*, and was decidedly higher than that of the evergreen conifer *Picea mariana*.

16. The rate of conduction under summer conditions was very high in comparison to that under winter conditions. This was less noticeable in the evergreen ericads than in other plants. The summer rate in *Chamaedaphne* was but 10–20 times that of the winter rate. The maximum rate obtained under conditions of experimentation which were not extreme was 23 cm. per hour in an evergreen ericad, while rates above 100 cm. per hour were frequently found in other shrubs and in herbaceous plants. An evergreen bog tree, *Picea mariana*, exhibited a distinctly lower rate of conduction than deciduous trees whether conifer or hardwood.

17. In the case of peat bog plants in nature, light (particularly sunlight) seems to be the effective factor in causing stomatal movements. Stomatal movements, while effective regulators of transpiration when they occur, do not appear so closely to regulate transpiration of peat bog plants as the evaporating power of the air.

18. In view of the fact that exposure to the very extreme summer conditions in 1911 and 1912 did not affect the vitality of the evergreen ericads, that neither did the average winter of 1910–1911, with its scanty snow covering during the coldest weather,

while the extreme winter of 1911–1912 killed the parts of the evergreen ericads which projected above the snow; and in view of the fact that the evergreen habit is hereditary, that the position of the leaves in winter is different from that in summer, and that the transpiration is decidedly less than that of deciduous shrubs and of herbaceous plants in summer but greater in winter, the xeromorphy of these plants is real xerophyty, occasioned fundamentally by the necessity of protection when exposed to winter conditions and used advantageously by these plants during the summer.

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# THE MORPHOLOGY OF ARAUCARIA BRASILIENSIS

## II. THE OVULATE CONE AND FEMALE GAMETOPHYTE

L. Lancelot Burlingame

(WITH PLATES XXV–XXVII AND TWO FIGURES)

In a previous paper (1), the writer has described the source of the materials used in this investigation and the methods used in their preparation. Nothing need be added to the details presented in that paper except to mention the fact that there have been wide variations of weather conditions here in the last four years during which these collections have been made. Considerable differences have been noted in the stages reached at the same dates during these years. The 1910 collections appear to be two weeks or more farther advanced than those of the next year. It is not easy to be sure of the facts in regard to this, for cones from the same tree taken on the same day often vary astonishingly. It may be, consequently, that the variations observed are purely fortuitous and would not be sustained if the observations were sufficiently numerous, although I am inclined to think they would be. If so, it would appear that a wet winter is decidedly favorable to early development. The collections of ovulate cones were made about once a week throughout three years for most of the seasons, but were made every day or two during the months of March and April.

Each ovulate cone is borne at the end of a short branch. From three to five such branches commonly occur at a single whorl of branches (text fig. 1). The rudiments of these cone-bearing branches and that of the central leafy shoot are formed within the terminal bud of a branch. They can be found by dissecting such a bud from which the daughter buds are beginning to emerge in early April or late March. I was unable to find any recognizable trace of them earlier. Although buds exist within such terminal buds earlier, I did not succeed in finding any means of distinguishing between cones and ordinary leaf buds until just before the swellings of the ovules make their first appearance.

The buds all look alike externally before this, and even on dissection are so much alike as not to be distinguishable. A branch that has borne a cluster of cones one year does not ordinarily

FIG. 1.—The tip of a fruiting branch bearing 6 young cones about 4 months old: the photograph was made about August 1; 4 of the 6 cones are borne on branches arising from the same whorl, the other 2 from the whorl below; the whorl just out of the bud consists of leafy branches only; $\times\frac{1}{3}$.

bear a crop the next season. A cone-bearing branch is usually thicker and looks more vigorous than a leafy branch in the spring.

When the cones have emerged from the terminal bud and are

clearly recognizable as such, they are about the size of an English walnut. They are now distinguishable from a leaf bud by their shape, by their lighter color, and by the more numerous and slenderer leaflike sporophylls. Pollination occurs at this stage. In 4 months they have reached the stage shown in text fig. 2. They are then 6–7 cm. long and about 5 cm. in diameter. The

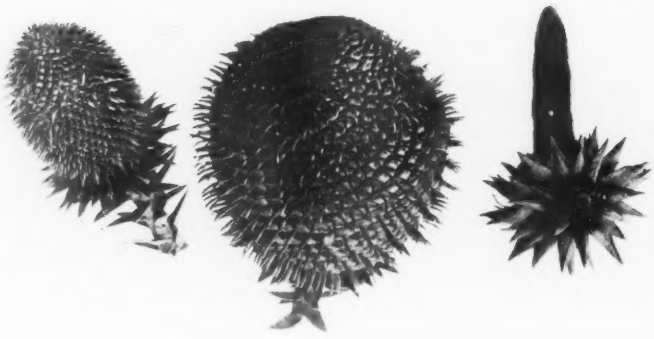

FIG. 2.—Cones of three different seasons, photographed in August: the smallest cone is 4 months old; the large cone is 16 months old; the cone axis with a few scales at base, 28 months old; the last shed its seeds in the preceding December and the largest cone would have shed seeds in the following December; $\times \frac{5}{14}$.

cone is rough and prickly from the turned-back tips of the sporophylls. The seeds are shed the fall or winter of the next year, when the cone has reached a length of 12–18 cm. and has shed many of its prickles. The life of an ovulate cone is thus somewhat less than two years. This is about a year less than that reported for *Agathis* (3), and also less than that of most conifers. There are usually 400–500 sporophylls on a cone (text fig. 2). They are arranged in steep spirals, making rather more than 1.5 complete turns.

Very soon after the cone is externally recognizable, the sporophylls show the first signs of the meristem that forms the ovule. At this time the sporophyll differs from a leaf in being differentiated into two regions. The outer part is slender and leaflike, while the basal portion is colorless, short, and stout, and tapers back slightly. The meristem is developed on the adaxial surface in a median position close to the base. It consists of many cells and grows rapidly. Figs. 1–3 show various views of it about this time. Although growth takes place throughout the sporophyll, yet four distinguishable meristems are established that determine its ultimate shape and form. The first of these is the meristem of the nucellus, already mentioned. As soon as it has made a beginning, another secondary meristem arises as a curved band across its upper surface. This curved meristematic band has its convex side directed toward the cone axis. By its growth is produced that part of the integument free from the scale. It is continuous below with the meristematic region of the scale located at its base. The fourth meristem is also a curved band, with its convexity directed toward the tip of the scale. It produces the ligule.

Not more than one sporophyll in twenty is fertile. Of those that do form ovules, many fail to mature them. Whether this tendency to sterility is equally marked in the native habitat of the tree I have not yet ascertained. It seems not unlikely that it may be due to the effects of cultivation in an alien habitat differing considerably from that of the highlands of Brazil. It has nothing to do with pollination, I am convinced, for all the cones are abundantly pollinated, both sterile and fertile sporophylls alike. The pollen tubes develop on the sterile ones, at least up to a length of 0.5 cm. or more.

The meristems very quickly produce the ovular structures. On account of this fact, and the further fact that one cannot distinguish sterile from fertile sporophylls until after sectioning them, I did not secure a complete series showing the development of the ovule. The outlines of the process, however, are clear. The basal meristem of the sporophyll elongates it, and the integument keeps pace. The result is a deep tubular cavity, deepest on the side next the sporophyll. At the base of this the nucellar meristem has been

elongating it to keep pace with the development of the integument. The result is shown in fig. 6. The line of union between the integument and the sporophyll proper is clearly indicated. In being attached along the entire side to the sporophyll, the ovule of *Araucaria* presents a sharp contrast to that of *Agathis*, which is attached only at the base (**6**).

In the nucellus three regions are at this time distinguishable. The tip consists of large clear cells, more or less isodiametric. Below this region the cells are elongated in the direction of the axis of the nucellus and arranged in fairly distinct rows. The rows become less definite toward the base. In the central portion of the base of the nucellus lies a group of cells with larger nuclei and denser contents. The megaspore arises in the midst of this group. This probably corresponds to the spongy tissue of various conifers, though it does not behave in precisely the same way during the further development of the ovule. Further reference to it will be made below.

The megaspore is picked out in a position above the bottom of the cleft between nucellus and integument. As it lies within the meristematic zone of the former, it follows that in the further growth of the ovule the female gametophyte will lie almost exclusively above the bottom of this same cleft. In this growth the nucellus does not enlarge its tip greatly. The result is that by the time the archegonia are ready for fertilization the nucellus is composed of a swollen base, containing the gametophyte, and a small extension above. The ovule at this time measures about 1 cm. in length and 3–4 mm. in diameter. The gametophyte extends through about half of it, and is about 1.5 mm. in diameter. It is somewhat oval in outline, with the archegonial end noticeably broader (fig. 4). The micropyle is shaped something like a human mouth, with its longer diameter transverse. Sometimes the upper lip is split back into a wide and deep V-shaped cleft extending back half the length of the gametophyte. Occasionally the latter breaks through the tissues of the nucellus and is openly exposed in the region of the archegonia. The further growth of the ovule gradually transforms the whole structure of ovule and sporophyll into the seed. The changes involved will be discussed

at another time. At maturity the gametophyte is 4–4.5 cm. long, 2 cm. wide, and 1.5 cm. thick. The entire seed structure is 5–6 cm. long, about 2.5 cm. wide, and 1.8 cm. thick.

The megaspore mother cell appears about the middle of May in the upper part of the group of denser cells already referred to. At first it differs only in size. As it enlarges, the adjacent cells show signs of disintegration. Fig. 5 shows a mother cell in synapsis. To the right and to the left of it may be seen two cells that are being flattened and whose nuclei are apparently beginning to degenerate already. I secured only a few preparations of the early stages and so cannot say certainly whether more than one megaspore mother cell ever begins development or not. Unfortunately I did not observe the reduction of the mother cell for the same reason. The chromosome numbers would indicate that a reduction does actually occur. Fig. 6 shows the position of the megaspore. In fig. 7 there may be seen two small cells just above the functional megaspore, that are probably the remains of the other members of the tetrad. A similar group is present in fig. 8. Very early the megaspore becomes vacuolate, with the very scanty cytoplasm forming an extremely delicate lining to the embryo sac. The nucleus is flattened, yet its thickness is several times that of the layer of cytoplasm in which it lies. It increases its volume, but does not immediately divide. Uninucleate embryo sacs are found in June, and binucleate ones (fig. 8) in July. By the latter part of the month as many as 64 nuclei may be present. Fig. 8 is that of a sac with two nuclei; in fig. 9 there are 8; and in fig. 10 a few less than 64. From this it would appear that the early divisions are simultaneous. As a matter of fact, I do not know that they are, for I have never observed a single mitosis in any of the more than 500 preparations of the free nuclear stages available for study. These preparations represent more than 50 collections of separate cones and two or three times as many separate ovules. It seems exceedingly strange that they should be so persistently missed. The same difficulty was encountered in a study made of the mitoses of the pollen mother cells (1). In that study I made the suggestion that those mitoses may occur at night. It has not yet been practicable to test out this hypothesis, and I mention it here merely

because I can think of nothing more likely. SAXTON (5) has since met the same difficulty and has hazarded the same guess. It may be that the mitoses are both simultaneous and passed through with extreme rapidity, and that it is merely chance that they have been missed. No indications of amitosis have been observed. Above the 64-nucleate stage the numbers are not regular, being usually somewhat less than the exact power of 2. Enlargement of the sac and multiplication of the nuclei continue up to the latter part of January. At this time there are more than 2000 free nuclei present. The cytoplasm still remains extremely scanty. Figs. 9–16 show the progress of development at intervals of about a month.

In January a change in the method of development occurs. Without any considerable increase of nuclei, the cytoplasm increases rapidly. As soon as it has become somewhat thicker, vacuoles make their appearance. The result (fig. 17) is that the central cavity is surrounded by a rapidly thickening sac of vacuolated protoplasm, with the nuclei largely confined to the inner border (fig. 18), In many cases the walls between the vacuoles break through. leaving the inner plasma membrane connected to the outer one merely by tenuous strands. The nuclei usually lie at the points where these strands join the inner plasma membrane. A few are found at the outer membrane, especially at the micropylar end of the sac. The protoplasm is thicker at this end also. This process goes forward very rapidly. The inner border advances on the central vacuole and the nuclei multiply somewhat. They now pass out along the cytoplasmic strands (figs. 19–21). By the time the inner border has closed up on the vacuole completely (fig. 20), most of the nuclei have migrated outward.

With the continued increase of the cytoplasm, most of it remains in the peripheral regions, especially near the micropylar end. It collects along the strands and plates until distinct uninucleate vacuolated sacs are formed. The nuclei are now generally suspended by still more delicate strands in the central portion of the sac. Fig. 22 shows how these sacs behave under the action of the killing reagents. Each sac appears to have its own wall of inclosing protoplasm capable of being separated from that of its neighbor. Mitoses now occur plentifully in preparations of the peripheral

portion of the prothallus. In this way there is formed a sort of colony of free cells closely packed together but yet capable of easy separation. This condition gradually passes into the vacuolated condition of the inner part of the gametophyte, in which region walls are not formed for some time. In fact, it never becomes so solid as the outer parts.

In about a month delicate walls have made their appearance between the peripheral cells (figs. 31, 32), though no definite cells have yet been organized centrally (fig. 25). The exact method of wall-formation was not made out. The process is remarkably suggestive of the way in which walls are formed in cleavage furrows in certain lower plants. One such cell is formed for each nucleus. After the formation of these first walls, the succeeding ones in the outer part are laid down on cell plates formed on the spindles in the ordinary fashion. The outer cells are generally uninucleate, while the central cells, after they have become walled off, become multinucleate by the time the archegonia are mature. They also later contain considerable quantities of starch.

In the further growth of the gametophyte, walls are formed on the spindles in the outer portion after each mitosis; in the central region wall-formation does not occur at this time. The transition from one region to the other is very gradual, and the walls thin out so gradually that it is almost or quite impossible to tell where there are actual walls. In fig. 31 are shown mitoses of both sorts. The one near the archegonium initial will have a wall formed on the spindle, while the one near the opposite border will not. Cell plates are formed in connection with the mitoses even before any walls are being formed on them (fig. 24). In the central region the nuclei are sometimes situated at the intersection of the larger strands and plates of cytoplasm and sometimes are suspended by much finer strands in the central region of the vacuolated spaces. Sooner or later these spaces, like the peripheral ones, form walls in the inclosing plates of cytoplasm and become cells. At the time of their inclosure by walls, they are commonly uninucleate. Later they frequently contain as many as 4–6 nuclei. Up to the time of the maturity of the archegonia the cytoplasm in all the cells of the gametophyte remains very scanty.

The outline of the growing prothallus may remain smooth and even (fig. 36) or become very irregular (fig. 35). The irregularity appears to be entirely due to two facts. The gametophyte, as already mentioned, is very delicate and plastic, and, in consequence, able to adapt its form to the cavity in which it grows. The second fact is that the cavity in which it is forming does not always enlarge regularly. Whether the irregularity is due in whole or in part to the action of the gametophyte is somewhat doubtful, as will be pointed out in the succeeding paragraphs. The development of the gametophyte beyond the fertilization stage will be further described in connection with the maturity of the ovule and the organization of the seed.

The cells surrounding the megaspore have already been mentioned as being larger, having larger nuclei and denser contents than other nucellar cells. They perhaps correspond to the so-called spongy tissue. The character of these cells is shown in figs. 6–8. As the megaspore enlarges, the innermost layers of these cells die and lose their contents. The walls also seem to disappear, though much more slowly. They are at first stretched out and closely compressed into a thick and compact band just outside the megaspore membrane. Inasmuch as this band of crushed cells does not appear to increase in thickness beyond a certain point, it seems reasonable to suppose that it is dissolved and perhaps used by the growing gametophyte. Immediately outside of this region of dead and empty cells there is a more or less distinct band of cells (figs. 10, 12, 13, 14) which stain more densely. This band of cells develops outward in advance of the gametophyte, keeping much the same relation to it as at first. The individual cells have more cell contents than the cells outside of the band. Their nuclei take basic stains strongly, as sometimes does the cytoplasm also. In short, they appear to be undergoing degeneration. The appearances described are such as have been observed in many other plants. The phenomena have been commonly ascribed to the effects of the gametophyte. It has been supposed that it secreted some sort of enzyme or other substance that diffused outward, killed and digested the cells, and prepared them for food for itself. It looks like a reasonable inference.

In the preceding paragraph I have described the appearance of the nucellar tissues around the growing gametophyte. I wish now to describe some of the anomalous conditions found that have led me to suspect the validity of the current accounts of the effects of the gametophyte on the nucellus. In fig. 26 is shown an apparently enlarging hole in the nucellus surrounded by the two usual bands of differentiated cells. Such ovules are fairly common. In some cases the megaspore membrane appears to be present. One might infer in such cases that the hole is the work of a gametophyte that for some reason or other has died. In most cases the megaspore membrane cannot be demonstrated; but since it is not well developed in any case, this would not appear to be an insuperable difficulty.

Fig. 27 shows a less common condition. There is no gametophyte here, nor is there any place for one; yet there is a remarkable correspondence in nucellar structure. In the center there is an enlarging mass of cells whose nuclei and cytoplasm are undergoing degeneration. The central cells are almost completely crushed; they have very little or no living contents. Outwardly the cells grade off through less and less crushed cells to a band of normal shape and size, but with densely staining contents, just as in the normal ovules. If there is no gametophyte and even no place for one, then the effects cannot be due to the presence of one. It seems that these facts admit of but one of two possible explanations: either the cells of the spongy tissue, which are possibly potential megaspore mother cells, are capable of producing the observed effects, or it is a quality of the nucellar cells themselves to behave in this fashion, regardless of the presence of a gametophyte or its antecedent archesporial tissue. A noticeable peculiarity of these cells in all cases is the thickening of their walls accompanying the death and disappearance of the protoplasm.

The development of such sterile ovules has nothing to do with pollination, apparently, for they occur regardless of whether the pollen has or has not sent tubes into the nucellus. They are relatively common and develop to advanced stages. Cones on unpollinated trees on the grounds of Stanford University develop to nearly normal size, though the gametophytes do not.

The megaspore membrane is usually thin and poorly organized.

It is variable in different ovules. In *Agathis* it is reported (3) that the megaspore membrane is thickest over the apex of the gametophyte and gradually thins out toward the archegonia in such a way as to allow the fertilization of the lower archegonia first and to protect from the pollen tubes the later maturing upper archegonia. There does not appear to be any such difference in *Araucaria*, though it is usually the case that the megaspore membrane disappears along with the band of dead cells in the region of the archegonia (fig. 4).

The time at which the pollen tubes reach the nucellus is subject to wide variations. They may do so as early as July or be deferred till late in the fall. The time at which they do so does not appear to exert any influence on the development of gametophyte or ovule, within the limits mentioned. So many tubes commonly reach and penetrate the nucellus that it is almost entirely destroyed. They usually enter through the tip, composed of large clear cells with little protoplasm, but may occasionally pass between the nucellus and integument for a very short distance before entering the former. In the cases of the large slitlike micropyles, through which the nucellus is exposed for a large part of its upper surface, the tubes ordinarily, at any rate, enter only through the tip. There do not appear to be any special peculiarities in the way the tubes penetrate the nucellus. They go fairly straight to the region of the archegonia. Occasionally one branches; a few strike the cap of dead cells over the apex of the gametophyte and then commonly turn aside. They are surrounded by a layer of dead cells somewhat like that around the female gametophyte, though it is less extensive and less regular. No indications of the breaking down of nucellar cells to make way for a pollen tube were observed unless the pollen tube was itself present to account for the effects. The nucellar cells below the tip commonly contain much starch, which largely disappears with the development of the tubes.

The uniformity with which the tubes enter the tip of the nucellus, even when a shorter and apparently more available path is present, suggests that they are attracted by a chemotactically active secretion from the glandular tip. I did not succeed in demonstrating such a secretion. There are often considerable quantities

of a slightly sticky liquid between the sporophylls, but I failed to find any evidence that it comes from the nucellar tip.

At about the time the walls are formed in the peripheral parts of the gametophyte, in the latter part of February usually, the archegonium initials become recognizable. Owing to their scanty contents they are recognizable only after they are somewhat enlarged. They vary in number from about 6 to 15 or more. They are situated in a ring around the crown of the prothallus. They do not all mature at the same time, though there does not appear to be any regular order. Commonly 5–8 mature and 3 or more of these are frequently fertilized. Within the circle the individual archegonia may stand alone (fig. 4) or they may be grouped in complexes (figs. 30, 44). Each archegonium is commonly surrounded by an individual jacket, though in some of the complexes there may be no cells at all between some of them (fig. 44).

The initial is commonly a wide U-shaped (fig. 31) or V-shaped (fig. 32) cell. It has a large nucleus and little cytoplasm. Sometimes a basal cell is cut off from this cell (fig. 33) before it becomes the actual initial. The nucleus of the initial divides and a periclinal wall separates a thin flat primary neck cell from the inner or central cell (figs. 32, 34, 37). The central cell enlarges much more rapidly than the neck cell (figs. 38–40). The latter soon divides by an anticlinal wall. This division is more frequently in the direction of its greater diameter. Each of the halves then divides into about 6 wedge-shaped cells. The nuclei of these wedge-shaped cells are invariably at the large end of the wedge. The points of the cells meet or nearly meet at the center of the neck. At this point the cells have commonly thinner walls, less cytoplasm, and a tendency to separate and leave a free passage to the egg (fig. 46). Viewed from above, the group of neck cells has either a rounded (fig. 46) or elliptic (fig. 47) outline; from the side they usually appear as a dome or cap (fig. 45).

Many variations in the form and outline of the neck occur. The size of the group as well as the number of cells in it is subject to considerable variation. The commonest arrangement is 12 cells arranged in a single tier (figs. 45, 46). Figs. 48–50 show three serial sections through a neck in which there is one extremely large cell.

The figures also show that the cells are not all in the same plane, but are placed more or less obliquely above one another. This is not unlikely due to crowding by the large cell. Many such asymmetrical necks occur in my preparations. Occasionally one cell is so large as almost to pass for a central cell. These large neck cells possess large nuclei, as may be seen from fig. 50. Occasionally there is more than a single tier of cells in the neck; fig. 51 shows a neck of 3 tiers of cells.

All the archegonial initials which I could certainly identify and all young archegonia occur in the surface layer of cells. In one preparation there were 4 cells in a row. The outer one resembled a primary neck cell. The lowest one was large and had the general appearance of a central cell. The inner of the other two was about one-third as large as the lowest and slightly larger than the second one. An imbedded archegonium might have resulted, possibly, from such an initial as this. Though the mature archegonia are very frequently displaced and overgrown by the neighboring cells, it is ordinarily easy to find the free open passages from them to the surface. I did not find any case in which it was not very probable that such a passage exists. I am fairly convinced, therefore, that there are no deep-seated archegonia in this species of *Araucaria*. I believe that all such appearances are due to displacement and overgrowth. EAMES has recently (3) expressed a somewhat similar opinion in regard to *Agathis*. I have examined a number of preparations of *A. imbricata* and have seen no deep-seated archegonia among them. The displacement and overgrowth of the archegonia is rendered very easy owing to the very delicate walls in the prothallus and to its frequently irregular outline. The more solid archegonium would thus be easily pushed into a position where the turgescent cells of the gametophyte would find an equilibrium of mutual pressures. The same fact would almost invariably lead to the crowding down into the archegonial cavity of the adjacent cells if they encountered any resistance at all in the expansion of the cavity in which the gametophyte grows.

The jacket usually consists of a single layer (figs. 42, 43) of more dense cells, which are usually uninucleate and with comparatively thin walls. The wall next the egg is somewhat thicker than the

others and is usually marked by a large thin spot, as is common among conifers. Occasional cells are binucleate, and sometimes the jacket is doubled in places (figs. 49, 50). In contrast to *Agathis* (3), the jacket is firmly united to the neck cells. In consequence of this the contents of the pollen tube pass through between the neck cells, in a manner to be described in another place.

The growth and development of the central cell resembles that of most other conifers in its general outlines. It enlarges rapidly but remains poor in cytoplasm (figs. 37–40). At first there is a single large vacuole, later there are many small ones in the more abundant cytoplasm (figs. 41, 42). At maturity there are usually no vacuoles and the cytoplasm is very dense (fig. 43). The nucleus enlarges with the development of the central cell. At first it is placed in the upper central part (figs. 39–41). It later migrates to one side (fig. 42) and nearer the neck. From here it passes to a position just below the neck (fig. 45) as if about to divide into egg nucleus and ventral canal nucleus. I was unable to find any evidence that it does divide. No mitotic figures were seen in the developing archegonium, nor were any nuclei, other than the one, ever seen before fertilization, except in one ovule. In this case a number of small nuclei were present in the upper part of what appeared to be a mature archegonium as yet unattacked by a pollen tube. It would be rash, perhaps, to assert that such a ventral canal nucleus is never cut off, even though a persistent hunt for it has failed to reveal it.

## Discussion

I shall not attempt at this time to discuss broadly the relationships of *Araucaria* to other members of the Coniferales, but merely to point out wherein it resembles some of them and in what ways it differs from all of them in certain features of its ovule and female gametophyte.

One of the first points in which this species of *Araucaria* (also *A. imbricata*) differs from *Agathis* is in the length of time taken to mature seeds from the first appearance of the seed cone. From the time it can be first recognized until the seeds fall is approximately 21 months. EAMES (3) reports *Agathis* as forming the

rudiments of its seed cones nearly a year in advance of pollination, while here there intervenes scarcely any time at all. In fact, in California the greater part of the pollen is likely to be shed before there are any ovulate cones to pollinate. Observations in the native habitat might show a different state of affairs and one more nearly paralleling that of *Agathis*. Other conifers of course are known in which the total time is even shorter than 21 months, but I am not aware of any one in which the time is distributed in the same manner.

The very considerable number of free nuclei before cell formation is another character that, taken with the very large gametophyte, reminds one of more primitive gymnosperms. Gametophytes of this size are known only among the Cycadales, Ginkgoales, and some taxads (as *Torreya*). A curious feature of its development is the strange absence of mitoses in the free nuclear preparations.

The manner in which the free nuclear stage passes into a gametophyte with walled cells is apparently different from that reported for any other plant. While there is a certain resemblance to the centripetal growth with "alveoli" reported for *Sequoia* (4) and others, yet the exact method is really quite different. The most essential feature of this difference lies in the delay of walls. If walls formed between the nuclei before the beginning of the centripetal movement of the inner border of the cytoplasm and were extended *pari passu* with it, and the nuclei divided to keep pace, there would be no very essential difference. While far too little is known about the exact methods of centripetal growth and wall-formation in any considerable number of genera to make any conclusions based on this feature more than tentative, it is clear that *Araucaria* need not be excluded from relationship either with podocarps or with abietineous conifers on this account.

While there is no proof that the gametophyte invades the nucellus in *Araucaria*, neither is it proved that it does so in other genera where similar appearances are commonly observed. The homology of the spongy tissue and its functions is none too clear anywhere. The peculiar glandular nucellar tip is found elsewhere only among conifers of podocarpineous affinities. THOMPSON has made the suggestion that the method of pollination found in the

araucarians is "proto-angiospermic." It seems to me, on the contrary, that we have been altogether too ready to accept the type of ovule which has a specialized pollen chamber securely hidden away at the base of the scale, and which can be reached by pollen only by means of special devices, as a primitive type. It is scarcely credible that the first step in the evolution of the ovule and seed should have been so complex. If, however, the gymnosperms possessing this complex type evolved a seed before a cone, as in fact there is good reason to think they did, then this might have been at least one of the earlier successful types. If, on the contrary, the cone was evolved before the seed, or simultaneously with it, as it may very well have been in an apparently simple cone, what would be more natural than that the pollen should lodge in any convenient place among the scales (sporophylls perhaps) of the cone? Such an ovule would have a much better chance of survival in such a cone than if exposed on the lobe of a fernlike leaf such as those possessed by the known Cycadofilicales. I am aware that I am thus attempting to introduce an apparently ancestorless conifer, but fail to see that a search for fitting ancestors is likely to be more difficult than deriving it from unsuitable ones.

Imbedded archegonia have been reported (**6**) for *Araucaria*, and a comparison made with *Sequoia* in which somewhat similar conditions are said to be present (**4**). My own observations do not bear out the presence of such imbedded archegonia in *Araucaria*. They are superficial in origin and become overgrown by the neighboring cells. SINNOTT has recently reported (**7**) practically identical conditions in the podocarps. The necks are not specially noteworthy, though they show a rather closer resemblance to those reported for *Podocarpus* (**7**) than to those of most other conifers.

The failure to find a ventral canal nucleus is somewhat surprising in so large a gametophyte, not having advanced beyond the evolutionary stage in other respects that has been attained by conifers generally. It seems more probable that it will yet be found. Ventral nuclei have not been found as yet in one species of *Torreya* (**2**).

The gametophyte, therefore, appears to be neither highly specialized nor exceptionally primitive in its structure. Its large

size and numerous and large archegonia are offset by the late development of walls and their persistent delicacy, by the apparent lack of a ventral canal cell, and by the rather specialized necks of the archegonia. Probably it presents more resemblances to the gametophytes of the Taxaceae and to those of the Taxodineae than to other conifers.

## Summary

1. The ovule possesses a very free nucellus with a glandular tip, a single integument adherent to the scale for almost its entire length, a ligule, a large micropyle, and spongy tissue surrounding the gametophyte.

2. There is probably a single functional megaspore, which develops into an embryo sac with about 2000 free nuclei before cell-formation.

3. Cell-formation follows on a peculiar centripetal growth of the cytoplasm and precedes wall-formation.

4. The first walls are formed on the surface of the free cells.

5. Secondary walls are formed on the spindles of the mitoses occurring in the primary cells of the peripheral regions of the gametophyte.

6. The outer cells are uninucleate, the inner ones are multinucleate.

7. The archegonia have single-tiered necks, usually, consisting of about 12 wedge-shaped cells.

8. The necks are on the surface of the prothallus but are often overgrown.

9. The archegonia may be single or occur in complexes and have a single-layered jacket.

10. A ventral canal nucleus may be absent.

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## EXPLANATION OF PLATES XXV–XXVII

FIG. 1.—Longitudinal section of a young cone in which the rudiments of the ovule are just becoming visible; ×12.

FIG. 2.—Longitudinal section of a slightly older sporophyll, showing the beginnings of the nucellus, integument, and ligule; ×15.

FIG. 3.—Longitudinal section through the meristem of the nucellus; ×62.

FIG. 4.—Longitudinal section of an ovule just before fertilization, showing the position and relative size of the gametophyte and a protruding nucellus; ×10.

FIG. 5.—Megaspore mother cell in synapsis; ×900.

FIG. 6.—Longitudinal section through an ovule in the latter part of June, showing the enlarging megaspore; ×20.

FIG. 7.—A megaspore just before division, surrounded by spongy tissue; ×62.

FIG. 8.—A binucleate embryo sac in late June; ×62.

FIG. 9.—An 8-nucleate sac in July; ×62.

FIG. 10.—A 64-nucleate stage in late July; ×62.

FIG. 11.—A 512-nucleate stage in late August; ×20.

FIG. 12.—A small portion of the parietal protoplasm and nuclei in late October; ×125.

FIG. 13.—A whole embryo sac in October; ×20.

FIG. 14.—An embryo sac in November; ×20.

FIG. 15.—An embryo sac in December; ×20.

FIG. 16.—An embryo sac in January; ×20.

FIG. 17.—The beginning of centripetal growth of the cytoplasm; ×20.

FIG. 18.—The micropylar end of the gametophyte shown in fig. 17; ×125.

FIG. 19.—Centripetal growth half complete; note that some of the nuclei are now migrating outward and that there are no indications of walls; ×20.

FIG. 20.—A detail of the same gametophyte as shown in preceding figure; ×62.

FIG. 21.—The completion of centripetal growth of the cytoplasm; ×62.

FIG. 22.—Detail from micropylar end of sac of age shown in preceding figure; ×125.

FIG. 23.—A slightly older gametophyte; ×20.

FIG. 24.—A detail from preceding figure showing lack of walls and presence of free cells; ×250.

FIG. 25.—A central part of the gametophyte in early March, showing the lack of walls; from the same slide as fig. 31; ×125.

FIG. 26.—Section through an ovule in which the nucellus is growing and the cavity enlarging, but in which there is no gametophyte; ×20.

FIG. 27.—Section through an ovule without any gametophyte, but with its place occupied by an enlarging mass of cells probably derived from the spongy tissue; ×20.

FIG. 28.—Early stage of the erosion of nucellus by the pollen tubes, July; note the abundant starch; ×62.

FIG. 29.—A nucellus in December when most of the starch has disappeared and all the upper part of the nucellus has been destroyed by the tubes; ×62.

FIG. 30.—Section through an archegonial complex; note the remains of the apical cap of dead cells and the superficial neck; ×85.

FIG. 31.—Micropylar end of thallus about March 1, with archegonial initial; ×125.

FIG. 32.—Lateral portion of another thallus with young archegonium near top; ×125.

FIG. 33.—An archegonium initial just before division; ×250.

FIG. 34.—Young archegonium just after cutting off primary neck cell; ×250.

FIG. 35.—Apical end of an irregular thallus with young archegonia; ×62.

FIG. 36.—Similar thallus with smooth outline and young archegonia; ×62.

FIG. 37.—Young archegonium; neck cell undivided; ×250.

FIG. 38.—Similar archegonium with neck divided; ×250.

Figs. 39, 40.—Enlarging archegonia; ×250.

FIG. 41.—Archegonium about one-half mature, showing neck, vacuolate cytoplasm, jacket, and position of nucleus; ×125.

FIG. 42.—Nearly mature archegonium with nucleus to one side and just below neck; ×125.

FIG. 43.—Mature archegonium; ×125.

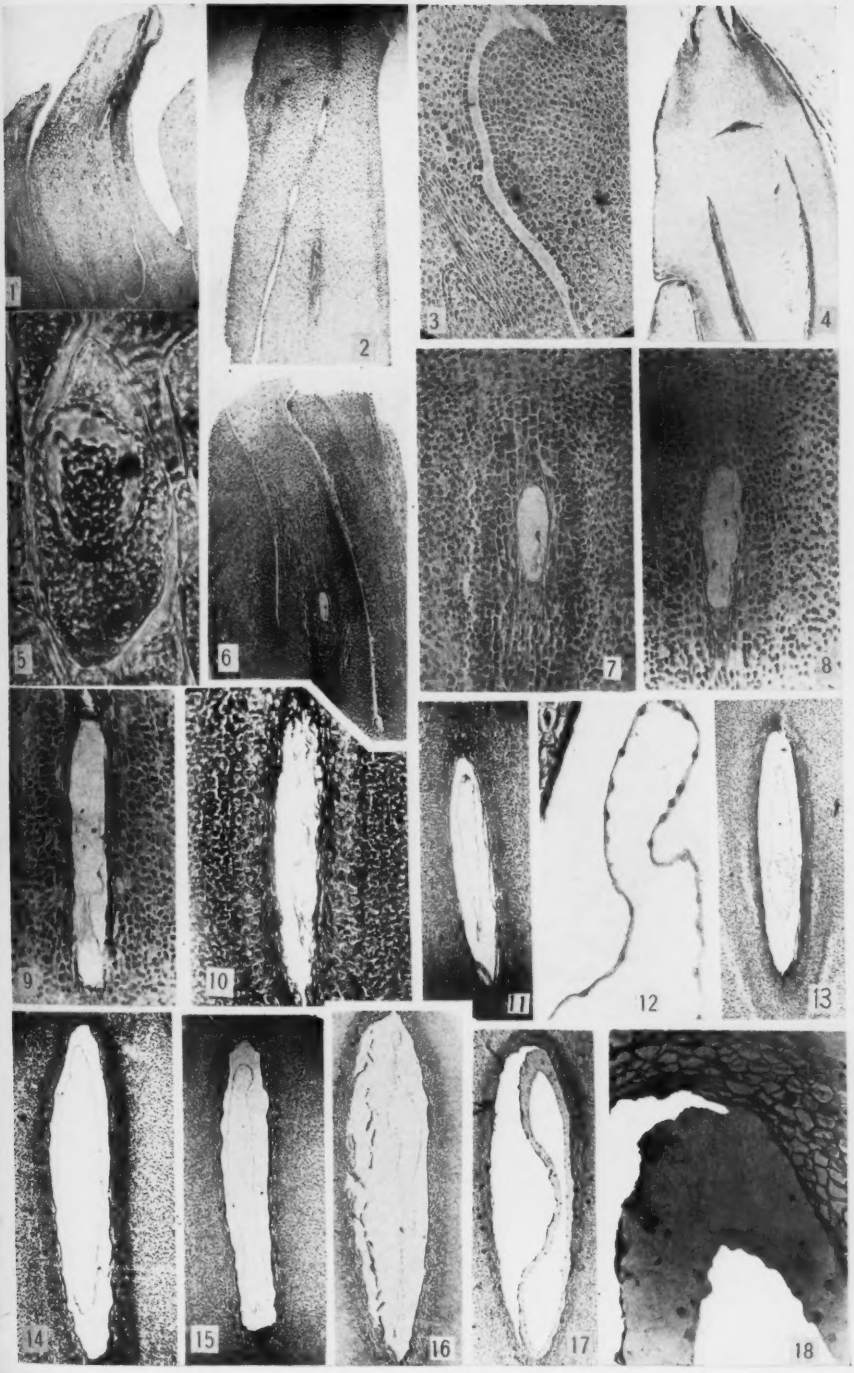
FIG. 44.—Cross-section through a complex where there are no jacket cells between two of the archegonia; ×125.

FIG. 45.—Section showing dome-shaped neck and position of nucleus below it; ×250.

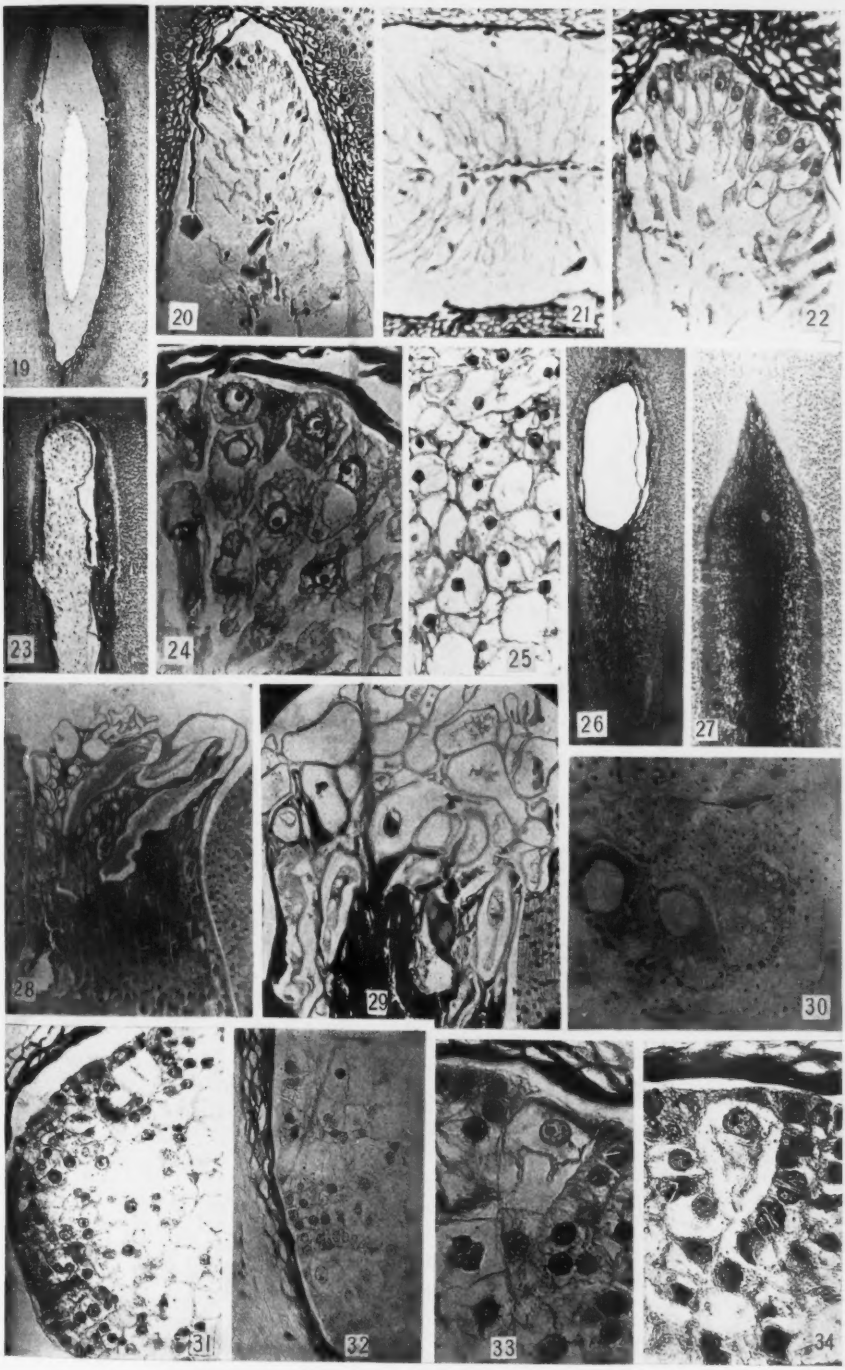
FIGS. 46, 47.—Cross-sections through usual type of neck; ×250.

FIGS. 48–50.—Serial sections through neck with one very large cell; ×125.

FIG. 51.—Longitudinal section of 3-tiered neck; ×250.




BURLINGAME on ARAUCARIA BRASILIENSIS

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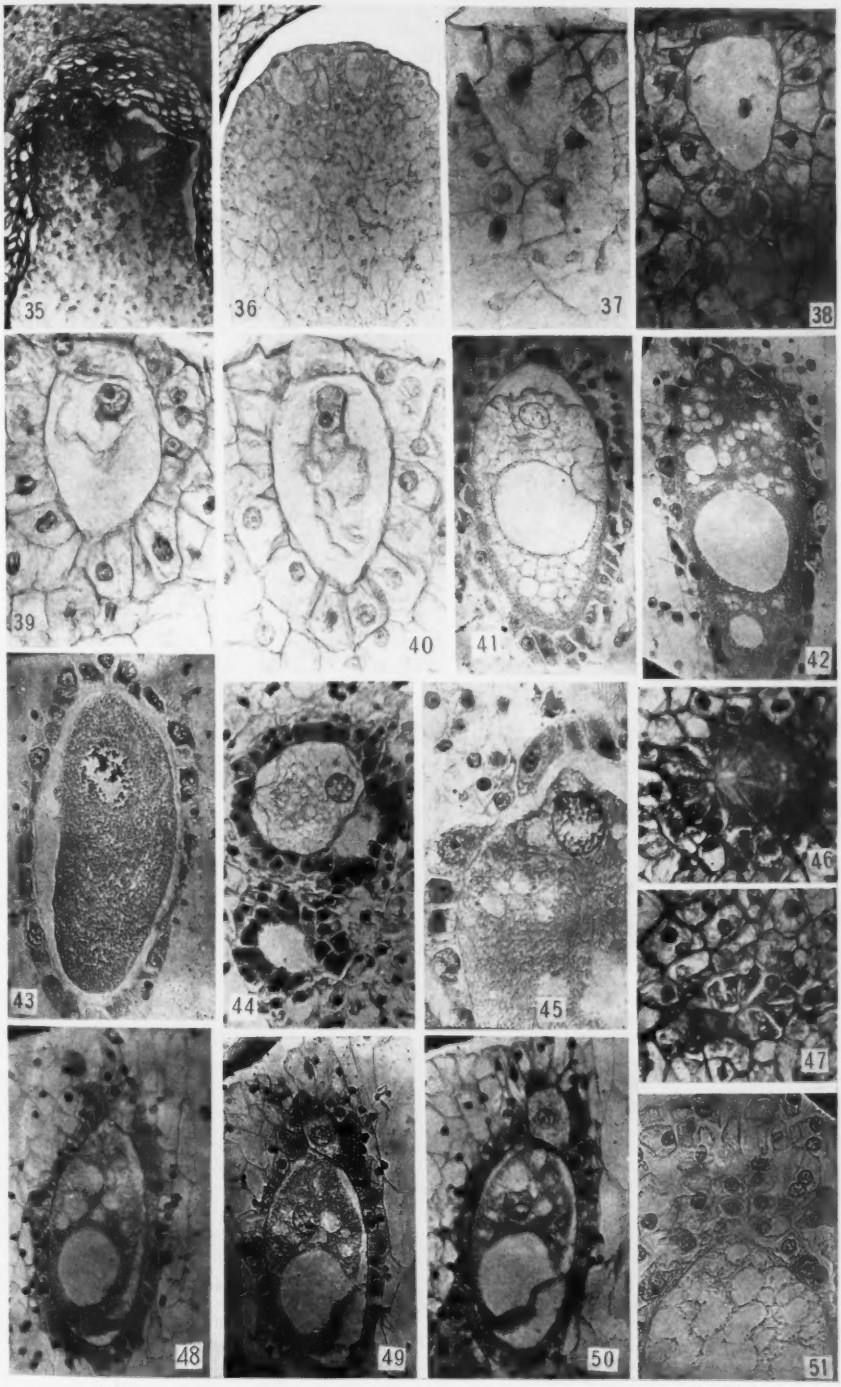



BURLINGAME on ARAUCARIA BRASILIENSIS

# THE ORIGIN OF MONOCOTYLEDONY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 187

JOHN M. COULTER AND W. J. G. LAND

(WITH PLATES XXVIII AND XXIX AND TWO FIGURES)

The origin of Monocotyledons from the Archichlamydeae seems well enough established to need no discussion in this connection. The evidence of vascular anatomy, supported by the historical record, as well as by general morphological considerations, seems to be explicit. It remained to obtain evidence of the transition from dicotyledony to monocotyledony. This seemed to be a peculiarly difficult situation, for it appeared to involve much more than the number of cotyledons. The difference in number was disposed of in two ways, the monocotyledonous condition being said to have arisen either by a fusion of the two cotyledons or by a suppression of one of them. Each of these views can be supported by a considerable body of evidence, based upon vascular anatomy and upon many intermediate stages in fusion or in elimination. The real difficulty in the situation, however, appeared to be in the fact that in Monocotyledons the cotyledon is a terminal structure, and in Dicotyledons the cotyledons are lateral structures. How could the terminal cell of a filamentous proembryo which had been producing a stem tip change its function and persistently produce a cotyledon? Any comparison of the proembryos of *Capsella* and *Alisma*, the two accepted types of Dicotyledons and Monocotyledons, emphasizes this difficulty.

A clue to this problem was furnished by the seedlings of *Cyrtanthus*, a South African genus of Amaryllidaceae, which was investigated by Miss FARRELL,[1] a graduate student of this department. In accordance with this suggestion, seeds of numerous Monocotyledons were obtained from South Africa and Australia, These were germinated and an abundance of material obtained for study.

[1] FARRELL, MARGARET E., Ovary and embryo of *Cyrtanthus sanguineus*. BOT. GAZ. 57:428–436. *pl. 24. figs. 3.* 1914.

The first case to attract our attention was that of *Agapanthus umbellatus* L'Hér., one of the South African Liliaceae. Although ordinarily monocotyledonous, a seedling was found with two well developed cotyledons. This discovery led us to hope that it might be used in determining the relation between monocotyledony and dicotyledony. The two conditions shown by the seedlings of *Agapanthus* are illustrated in fig. 1. If the seedlings of the same species are thus indifferently monocotyledonous or dicotyledonous, there must be some evident relationship between the two conditions.

In the following account no attempt will be made to cite the literature of the subject. It is sufficiently well known to students of Angiosperms, and most of it is available in COULTER and CHAMBERLAIN'S *Morphology of Angiosperms*. When the remaining material that has become available is investigated, a more detailed account of the whole subject of the development of cotyledons will be given.

The structure of the monocotyledonous seedling of *Agapanthus*, a photograph of which is reproduced in fig. 1, is shown by the series of transverse sections given in figs. 2–13, and by their diagrammatic reconstruction in fig. 14. The section through the cotyledon and first leaf above the cotyledonary sheath (fig. 2) shows three vascular strands in the cotyledon, arranged in a triangle, with the xylem directed toward the center. If the middle strand had not been laid down, the two laterals would show the inverse orientation that has suggested that the monocotyledonous condition has arisen by a fusion of two cotyledons. The cotyledon above the sheath is cylindrical, but as the sheath is approached it begins to invest the first leaf (fig. 3). Soon the sheath becomes a complete ring in transverse section, which increases in thickness around the leaf as the transition region is approached, until, at the junction with the first leaf, the investing sheath becomes of almost uniform thickness (fig. 9), that is, the side of the sheath away from the cotyledon is as thick as the side which is continuous with the cotyledon. In all the many seedlings sectioned, the cotyledonary sheath is a simple ring in transverse section, without any infoldings.

The three strands retain their positions with reference to one another until just above the transition region, where they approach

one another, and their phloem strands become united (fig. 9). Farther down the three cotyledonary strands unite completely, and the phloem divides (fig. 10). The single bundle of the first leaf turns inward (figs. 9 and 10) and unites with the cotyledonary strands (fig. 11). The lateral bundles of the leaf are disregarded.

FIG. 1.—Monocotyledonous and dicotyledonous embryos of *Agapanthus umbellatus;* ×4.

since they appear late, and in early seedling stages are not connected with the main strand or with the cotyledonary plate. This union of strands, three from the cotyledon and one from the first leaf, forms a siphonostele (fig. 11), which is the "cotyledonary plate." The strand from the first leaf continues into the hypocotyl and becomes one of the poles of the root, and from the cotyledonary strands that enter into the structure of the cotyledonary plate, two other strands pass down the hypocotyl to form poles of the root,

which is therefore triarch. Farther down, the strand which has come from the first leaf forks, and the root becomes tetrarch. Older seedlings were not examined, but probably further forking of strands occurs, making the root eventually polyarch.

In thus describing the course of the vascular strands, the sequence given seems to be that of their formation, although we did not observe the procambial strands. If this be true, they are laid down first in the growing cotyledon and first leaf, become united later in the cotyledonary plate, which then gives rise to the root poles. This means that the vascular strands do not determine the development of the structures of the seedling, but that the primordia of cotyledon and first leaf start and the vascular strands are laid down in the growing organs. In other words, growing primordia determine the vascular strands rather than the reverse. If the cotyledonary sheath is very massive, vascular bundles may be laid down, and these may have no connection with other bundles. In the cotyledonary sheath of *Doryanthes Palmeri*, which is very thick, a vascular strand was found at a point opposite the massive cotyledon. This solitary strand had no connection with the cotyledonary plate, or with any other strand, beginning and ending blindly in the sheath. The inference seems evident that vascular strands are secondary structures, whose appearance is dependent upon the character of the primary structure, and therefore of no phylogenetic significance in the seedling.

It is noteworthy also that in the seedling of *Agapanthus* there is no stem primordium. In fact, the stem is a very belated organ, not having appeared in the seedlings under investigation. When and how a stem primordium is organized later was not seen. Certainly in the well developed seedlings of *Agapanthus* there is no stem primordium that gives rise to lateral leaf primordia. All of the meristematic tissue is involved in cotyledon and leaf-formation.

The structure of the dicotyledonous seedling of *Agapanthus* shown in fig. 1 is indicated by the series of transverse sections given in figs. 15–28, and by their diagrammatic reconstruction in fig. 29. The seedling is of the same age as the monocotyledonous one. The two cotyledons are the same length, but one is slightly thicker than the other (figs. 15 and 16). The cotyledonary sheath extends

farther upward on the side opposite the first leaf, giving the seedling a slight asymmetry (fig. 17), but soon the sheath becomes a symmetrical ring in transverse section (figs. 18 and 19). Each cotyledon has two lateral strands, as if the middle one present in the monocotyledonous seedling has not been laid down. The second leaf is also well developed, so that six strands approach the cotyledonary plate (figs. 21–23). These gradually converge, the phloem of the cotyledonary strands uniting with that of the first leaf (fig. 23), and lower down with that of the second leaf (figs. 24 and 25), but the xylem does not fuse until farther down (fig. 26). As in the monocotyledonous seedling, the cotyledonary plate is a siphonostele, and the strand of the first leaf continues directly as one of the poles of the triarch root, and lower down divides, resulting in a tetrarch root.

So far as the vascular strands are concerned, the two seedlings differ in the number laid down in the cotyledons. The larger single cotyledon contains three strands, while each of the smaller cotyledons of the dicotyledonous seedling contains two strands. The organization of the cotyledonary plate and of the root poles is the same in both cases.

It is obvious that this difference in the number of vascular strands does not determine the development of one or two cotyledons; the number of strands is simply a result of the development of one or two cotyledons; for vascular strands are differentiated in the tissues of a growing organ. It seems clear, therefore, that the appearance of one or two persistently growing points in the cotyledonary region of the proembryo determines the monocotyledonous or dicotyledonous condition; and that in *Agapanthus* the number of such growing points is variable.

In comparing the two seedlings, a suspicion might arise that the so-called dicotyledonous seedling, with its two leaves as well as its two cotyledons, is a case of the fusion of two embryos, or rather two produced by a single proembryo. This possibility has not been traced through in detail, but the vascular situation just described shows that the dicotyledonous seedling is merely a slight modification of the monocotyledonous one, and gives no suggestion of the "fusion" of two embryos.

It seems evident that the variability in the number of cotyledons could appear only in massive proembryos. The conception of monocotyledonous and dicotyledonous embryos has become a somewhat rigid one because it has been based upon such proembryos as those of *Alisma* and *Capsella* as types. It has become customary to regard these filamentous proembryos as primitive and typical of the two groups, and the massive proembryos that occur in both Monocotyledons and Dicotyledons as modifications. It is our belief that massive proembryos represent the primitive condition of proembryos in Angiosperms, and that only from such a proembryo could the monocotyledonous and dicotyledonous conditions have differentiated. After this differentiation, the difference has become relatively fixed by the reduction of proembryos to filaments. While massive proembryos occur in all the three great divisions of Angiosperms, they are notably present among the Ranales, from which the monocotyledonous branch seems to have arisen; and they are also retained by many of the Monocotyledons, notably the Arales and Liliales, and in these groups one may expect to find occasional dicotyledony or even polycotyledony.

The sequence of events in the development of the embryo of *Agapanthus* is as follows. As the massive proembryo enlarges, the root end elongates, thus remaining narrow and pointed; while the shoot end widens, becoming relatively broad and flattish. At this broad and flat end the peripheral cells remain more actively meristematic than do the central cells. It is this peripheral meristematic zone that is the cotyledonary zone. In this zone two more active points or primordia appear and begin to develop. Soon the whole zone is involved in more rapid growth, resulting in a ring or tube, but with the primordia still evident. The sequence of these two stages must be kept distinctly in mind, namely, (1) the appearance of primordia, and (2) zonation. The cotyledonary zone continues its growth until a tube of considerable length is developed, leaving the apex of the proembryo depressed. In the case of the dicotyledonous embryo of *Agapanthus* the two primordia on the rim of the tube continue to develop equally, the growth of the whole cotyledonary zone being shared equally by the two cotyledons. In the

case of the monocotyledonous embryos, the cells of one of the cotyledons gradually lose their meristematic activity, but those of the other one continue division, and as a result the so-called single cotyledon is developed, which is really the growth of the whole cotyledonary zone under the guidance of a single growing point. One cotyledon is not eliminated, but the whole growth is diverted into one cotyledon. Of course "tube" or "sheath" and "cotyledon" are all one structure, arising from the cotyledonary zone. As a result of the checked growth of one of the cotyledonary primordia, there soon develops the appearance of an "open sheath" and a "terminal" cotyledon. The developmental stages in this case, therefore, are first two cotyledons, and then, because of one-sided growth, one large cotyledon and one so small as to be easily overlooked.

The two primordia appearing upon the cotyledonary zone mark the places where vascular strands will be laid down. It would seem that the positions of vascular strands in such a case, therefore, are of no significance beyond indicating possibly the places where primordia have appeared, and also sometimes suggesting the number of primordia; but they cannot be used even for these purposes with absolute certainty.

It would be interesting to assemble the many cases of unequal cotyledons and note the variation in the checked growth of one of them, so that it ranges in appearance from a mere protuberance or ligule-like appendage, to a fairly well developed, but smaller, cotyledon. Such a series can be observed among the so-called pseudo-Monocotyledons. In such a series it would be noted also that if the checked growth of one of the cotyledons is very early, it will contain no vascular bundles, all the cotyledonary strands being laid down in the growing cotyledon. In many cases, as in *Cyrtanthus*, as growth is diverted from one cotyledon to the other, the procambium strands are also diverted toward the larger and later functioning cotyledon, and finally the strands unite. This phenomenon of fusing strands has been interpreted as an evidence of the fusion of cotyledons. In *Fourcroya Bedinghausii*, however, we found that the procambium strands of the primordium which represents a second cotyledon do not drift across and unite with the

strands of the single functioning cotyledon. In these cases, therefore, one cotyledon is developed whether opposing strands unite or not. In monocotyledony, therefore, as shown by *Agapanthus*, the number of vascular strands in the single cotyledon is likely to be greater than it would have been if both cotyledons had developed, for the cotyledon itself is larger. It is such cases that have suggested that monocotyledony has arisen by the suppression of one cotyledon. It is not so much the suppression of one cotyledon, as the growth of the whole cotyledonary zone to form a single cotyledon. In other words, in such a case a cotyledon is no more suppressed than are petals in Sympetalae.

A further examination of the proembryos of *Cyrtanthus sanguineus*, reported upon by Miss FARRELL (*loc. cit.*), shows that in this species four primordia may appear upon the cotyledonary zone, which for a time develop equally. Then the whole zone becomes involved in the more rapid growth, giving rise to the cotyledonary ring or sheath, but with the four growing points still prominent. In the next stage the cells of the ring between a pair of growing points on each side become more active, and the four original growing points begin to "grow together" in pairs, so that two cotyledons, each with two points, begin to appear. During this stage, which may be called figuratively a "fusion in pairs," the cotyledonary sheath still continues to elongate. Later on one of the two cotyledons begins to develop faster than the other, resulting in two unequal cotyledons, which are connected at base by the thick ring. Gradually the cells of the smaller cotyledon cease dividing, and, those of the other continuing to divide, the result is a seemingly single terminal cotyledon. The developmental stages in this case are four cotyledons, two cotyledons, and finally one large cotyledon, associated with another one so small as to escape ordinary observation. The suggestion here of the possibility of polycotyledony in *Cyrtanthus* is plain, and the explanation of the polycotyledonous condition among certain Gymnosperms seems evident.

In the current accounts of the embryogeny of *Sagittaria* and of other forms with filamentous proembryos, the development of the proembryo from the filamentous condition to the organization

of growing points has not been traced, and the general conclusion has been reached that the terminal cell forms the single cotyledon. An examination of the stages between the filamentous proembryo and the apparently terminal cotyledon has shown that this conclusion has been taken for granted rather than tested. It is obvious that a massive proembryo is formed before growing points appear. The terminal cell of the filamentous proembryo of *Sagittaria* develops a mass of tissue whose meristematic peripheral cells develop a ringlike cotyledonary sheath, which in the growing seedling is still recognizable as an extremely narrow ring opposite the functional cotyledon. Just within this sheath, opposite the functional cotyledon, is a plate of cells, one or two cells thick, occupying the site of the second cotyledon which started as a second growing point in the cotyledonary zone. This plate merges with the base of the sheath and the first leaf, and in one instance was observed to extend upward 160 $\mu$, and ended in two points (fig. 30). This vestigial structure may be interpreted variously, but it seems most natural to regard it as a vestige of the second cotyledon. In *Sagittaria* and *Alisma* the "growing point" of the stem has been traced to a definite plate of cells beneath the cotyledon. This plate of cells, however, is not the stem primordium, but the primordium of the first leaf. The "notch" so characteristic of these embryos is developed by the checked growth of a cotyledon primordium on one side of the cotyledonary sheath, and at the base of this notch, which is really between two cotyledon primordia, the first leaf develops.

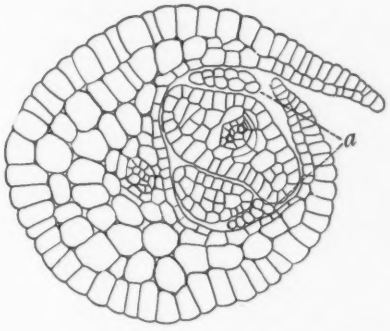


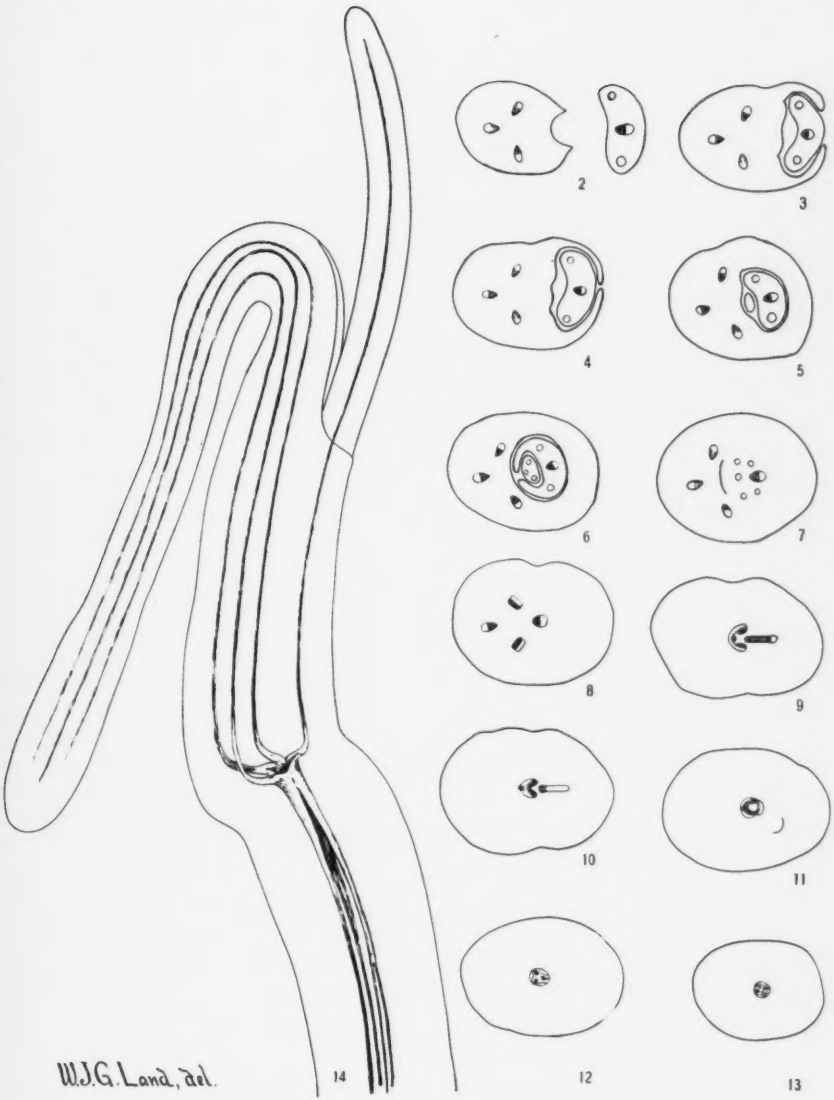

FIG. 30.—*Sagittaria variabilis:* transverse section immediately above the cotyledonary ring; *a*, rudiments of second cotyledon (?); ×150.

The conclusion is that in both Monocotyledons and Dicotyledons a peripheral cotyledonary zone gives rise to two or more

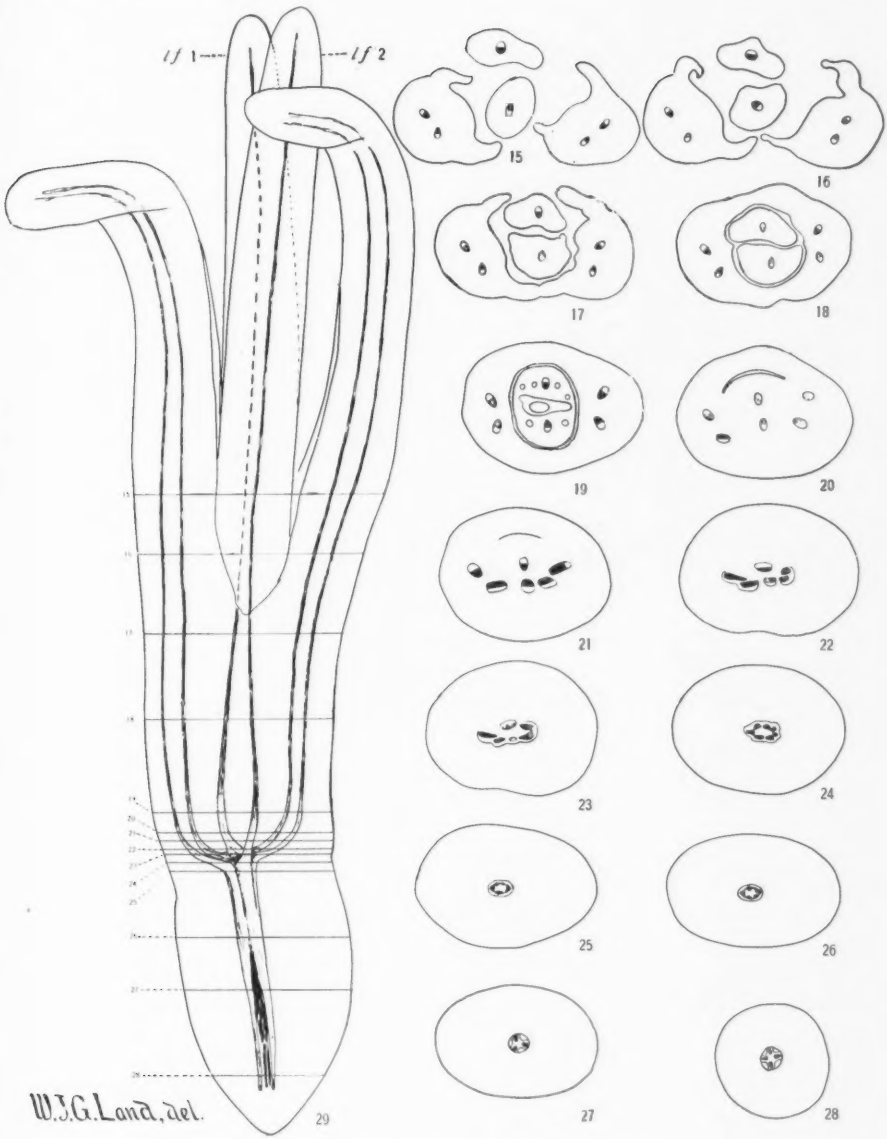
growing points or primordia, and that this is followed by a zonal development resulting in a cotyledonary ring or sheath of varying length. If both growing points continue to develop equally, the dicotyledonous condition is reached. If one of the growing points ceases to develop, the growth of the whole cotyledonary zone is associated with that of the other growing point, and the monocotyledonous condition is reached. In other words, monocotyledony is not the result of the fusion of two cotyledons, or of the suppression of one; but it is simply the continuation of one growing point on the cotyledonary ring, rather than a division of the growth between two growing points. In the same way polycotyledony is the appearance and continued development of more than two growing points on the cotyledonary ring. In fact, in *Cyrtanthus* four growing points appear at first, which under certain conditions might result in four cotyledons. The whole situation has its parallel in sympetalous corollas, in which there is zonal development associated with three, four, or five separate growing points, which, continuing development, are recognized as petals.

It follows that cotyledons are always lateral structures arising from a peripheral cotyledonary zone at the top of a more or less massive proembryo. This reduces cotyledony in general to a common basis in origin, the number of cotyledons being a secondary feature. The constancy in the number of cotyledons in a great group is no more to be wondered at than a similar constancy in the number of petals developed by the petaliferous zone.

The organization of the stem tip in the seedling is worthy of consideration. In the mature seedlings of *Agapanthus* there is no appearance of a stem tip; all of the meristematic tissue at the tip of the proembryo is involved in the peripheral cotyledonary apparatus and the centrally placed leaves. The stem can be regarded as existing only hypothetically in the siphonostelic cotyledonary plate. Later in the history of the seedling the central region of the embryo beneath the leaves elongates and the stem structure begins to appear. If the early leaves of a plant are very small, an organized stem tip appears earlier in the history of the embryo, but it is doubtful whether in Monocotyledons any stem structure appears until late in the history of the seedling. In fact,




COULTER and LAND on AGAPANTHUS




COULTER and LAND on AGAPANTHUS

the early and vigorous development of the first leaf in Monocotyledons is probably associated with checking the growth of the adjacent cotyledonary primordium.

Incidentally the late appearance of the stem as an organized structure probably enters into the explanation of the fact that the stem is the most advanced organ of the body in structure, as vascular anatomy has indicated repeatedly. In any event, the cotyledonary strands and first leaf strands organize the cotyledonary plate, which in turn gives rise to the root poles, and later determines the character of the stem cylinder.

The main thesis of this study of cotyledony, however, is to release it from its rigid morphological categories, by showing that the cotyledonary apparatus is always the same structure, arising in the same way, and varying only in the details of its final expression.

UNIVERSITY OF CHICAGO

## EXPLANATION OF PLATES XXVIII AND XXIX

FIGS. 2–14.—Monocotyledonous embryo of *Agapanthus umbellatus:* figs. 2–13, transverse sections beginning just above the cotyledonary sheath and ending in the cotyledonary plate (the sequence of numbers indicates the sequence of sections); fig. 14, a diagrammatic reconstruction of the sections; ×14.

FIGS. 15–29.—Dicotyledonous embryo of *Agapanthus umbellatus:* figs. 15–28, transverse sections beginning just above the cotyledonary sheath and ending in the cotyledonary plate (the sequence of numbers indicates the sequence of sections); fig. 29, a diagrammatic reconstruction of the sections; ×14.

# A METHOD OF CONTROLLING THE TEMPERATURE OF THE PARAFFIN BLOCK AND MICROTOME KNIFE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 188

W. J. G. LAND

(WITH TWO FIGURES)

The successful production of a continuous ribbon of paraffin depends chiefly on the temperature of the block in which the object is imbedded, and of the microtome knife. The hardness and size of the object may of course affect the ribbon, but if the object is small, not refractory, and completely infiltrated, the effect is practically negligible. If the temperature of the knife and block is made sufficiently low, very thin sections can be cut without unduly compressing the object, regardless of the melting point of the paraffin.

With paraffin of a given melting point there is a definite temperature at which the block and knife must be kept in order to produce a continuous ribbon of definite thickness without injurious compression of the sections. If the microtome is set for thinner sections without lowering the temperature of the knife and block, the sections are compressed more and more as they are made thinner, until a point is reached where the tissues are crushed out of all resemblance to their original condition. This crushing of the sections has in some instances resulted in erroneous interpretation of structures even by otherwise competent investigators. Again, if the microtome is set for thicker sections and the temperature of the knife and block is not correspondingly raised, excellent sections result, but their edges refuse to weld and they come away singly, a source of much trouble when an absolutely unbroken series is required. If the thickness is further increased, the sections come away rolled so tightly that in some instances they cannot be unrolled even by floating on warm water.

In practice, when thick sections (10–20 $\mu$) are wanted, the object is imbedded in paraffin melting at 45–52° C. If thinner

sections are required (3–5 $\mu$), paraffin melting at 58–62° C. is necessary. Even when the harder paraffin is used, in order to cut sections 2–4 $\mu$ thick, at ordinary room temperature, the knife and block must be cooled. If they are not cooled below the room temperature, which in winter is usually about 20–22° C., the sections are hopelessly crushed. In summer it becomes increasingly difficult to make thin sections.

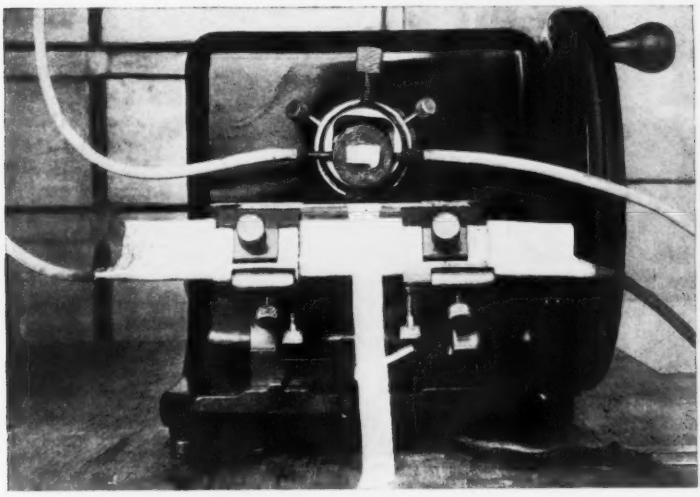


FIG. 1.—Apparatus for temperature control, showing object holder and cooling trough in place.

Many delicate plant tissues, notably certain liverworts, can be imbedded in paraffin melting at 45–52° C. with perceptibly little injury, but when the same material is imbedded at a temperature of 58–62° C. serious injury may result. For example, a tropical species of *Notothylas*, which at 52° showed little shrinkage, at 62° shrunk to nearly half the original thickness of the thallus. In addition to the action of heat, the effect of the large coefficient of expansion of paraffin (0.00027854) becomes marked when the harder paraffin is used. It follows that by using paraffin of a low melting point, the effect of heat and of the coefficient of expansion can be minimized.

In this laboratory the practice has been to cool the knife and block with lumps of ice. This method, while in the main giving good results, is unsatisfactory for the finest sectioning. Water usually gets on the ribbon and does damage; also great care is necessary to keep the microtome from rusting.

The various difficulties were in a large measure overcome as

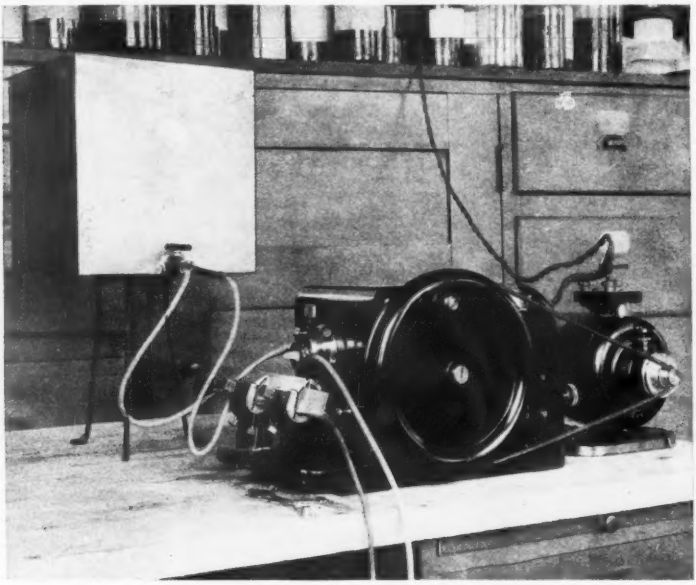


FIG. 2.—Apparatus for temperature control, showing all attachments

follows: The face of the ordinary circular metal object holder, which comes with all microtomes of the better class, was turned out to form a cup. A plate of thin brass was soldered over the cup, making a new face for the object holder, and turned down to the original diameter of the disk. Two holes were drilled and tapped at opposite sides of the disk and two short tubes for attaching small rubber tubes were screwed in. Fig. 1 shows this disk in the clamp of the microtome.

For controlling the temperature of the knife, a trough of thin metal—tin, brass, or copper—about 20 cm. long and about 2 cm.

deep was made to take in the knife and to permit about 1 cm. of the knife to project. A nipple at each end of the trough serves to attach small rubber tubes (fig. 1). The ends of the trough should be soldered to prevent leaking on the microtome. Any microtome knife having a detachable handle can be used. A flexible safety razor blade may be used, provided the heads of the clamping screws are flush with the sides of the blade holder.

A tank provided with a stopcock and Y-tube is placed above the microtome at a height which will insure a good flow of water. One tube of the "Y" is attached to a tube of the object holder by a rubber tube of small caliber, the other to the nipple of the cooling trough. Tubes lead from the holder and from the trough to a waste receptacle. The tank is filled with water of the proper temperature for the required thickness of ribbon. The block and knife reach the proper temperature for cutting soon after the water is turned on. The entire apparatus is shown in fig. 2.

The apparatus was designed primarily for cutting very thin sections (2–4 $\mu$) of liverworts which had to be imbedded in soft paraffin, but it has been found useful when very thick sections (20–50 $\mu$) are wanted. In cutting the latter, the knife trough is detached, and the tank filled with warm water which is allowed to flow through the object holder only. The temperature of water necessary for cutting sections of various thicknesses is easily determined by experiment.

University of Chicago

# BRIEFER ARTICLES

## SUCCESSFUL ARTIFICIAL CULTURES OF CLITOCYBE ILLUDENS AND ARMILLARIA MELLEA

(WITH THREE FIGURES)

During the course of some culture work with the wood-destroying polypores in the fall of 1913, it was found of interest to try out similar methods with an agaric form. Spores were obtained from a fungus that at the time was identified as *Clitocybe illudens*, and from it dilution cultures were made on a beef-malt-agar medium. The spores were found to germinate readily, and in the course of three or four days numerous separate colonies appeared on the agar surface. No evidence of contamination being visible, separate colonies were transferred to sterile culture tubes of the same medium on November 15. Vigorous growth took place and the tubes soon displayed thick felts of a brownish-white mycelium.

FIG. 1

Early in December a small dark brown area was noticed in one of the cultures, which soon gave rise to several dark brown finger-like papillae. These continued to elongate, and lighter colored, somewhat more slender regions appeared at their tips. These now enlarged rapidly and soon

took on the "button" form of the young fruiting agaric. On December 26, the accompanying photograph (fig. 1) was taken, and four days later the pilei had opened to the mature condition shown in the second photograph (fig. 2). As will be seen, the fruit bodies developed in a quite normal manner, and, except for their size and the somewhat "recurved" condition of the pilei, appeared to be quite normal. Fruit bodies examined with the microscope were seen to be sporulating profusely, and the spores were found to be quite normal as to color, shape, and size for this form. Upon being transferred to the beef-malt-agar medium, the spores germinated quite as readily as those from the original fruit bodies. Cultures are at present being maintained with the hope of obtaining a second fruiting generation, but as yet (March 14, 1914) no indications of the formation of fruiting bodies of the second generation have been observed.[1]

FIG. 2

The fact that the formation of normal fruit bodies on a synthetic medium is somewhat rare led me to consider what possible conditions may have effected this result. Cultures were found to fruit in either light or darkness, and so the presence or absence of light as a factor seemed to be eliminated, although it must be said that the first stages were always initiated in the dark. All cultures from the same fruit body made on one particular lot of medium, which had been slightly

[1] Since the preparation of this article, numerous fruiting bodies of the second spore generation have been obtained, showing such striking variations in form from the original parent that it has been thought best to discuss this phase in a future paper.

scorched in preparation, either fruited or produced abortive fruit bodies, while no fruiting was observed in other cultures. The scorched condition of the medium would of course give rise to substances not generally present in the culture medium, and it is suggested that this condition of nutrition may have been the determining factor. It is also of interest to note that the fruit bodies from which the spores were obtained were frozen solid when collected, which did not appear to injure the viability of the spores.

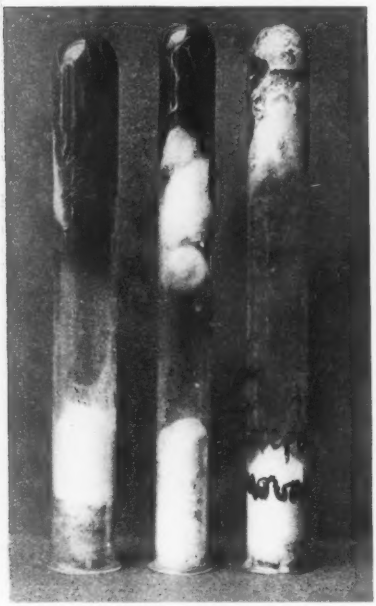


FIG. 3

Cultures of another very interesting agaric were obtained from the pathologist of the Forest Products Laboratory at Madison, Wisconsin, namely *Armillaria mellea* Vahl. This species forms a whitish mycelium which soon turns to a dark brown. The interesting feature, however, is the formation of the so-called "rhizomorphs." These are described as appearing in nature as shining black strands often resembling the roots of the host. They appear soon after inoculation on agar cultures, ramifying throughout the substratum. Here, however, they are of a shining light gray color, and are flat and ribbon-like, often branching dichotomously (fig. 3). Upon pentrating to a free surface these rhizomorphs immediately give rise to the ordinary vegetative mycelium.—V. H. YOUNG, *University of Wisconsin, Madison.*

## THE AMOUNT OF BARE GROUND IN SOME MOUNTAIN GRASSLANDS

In July 1911 the writer staked out a series of 19 quadrats for study of the grassland of a mountain park at Tolland, Colorado. In that year collections were begun and censuses of some of the quadrats made. The plan was adopted of estimating at intervals the percentage composi-

tion of each quadrat. Early in 1912 more detailed work was undertaken, all findings being recorded on catalogue cards. Each time a quadrat was examined an e timate[2] was made of the percentage of bare ground. Since there seem to be no published records of such studies in our mountains, it has occurred to the writer that a brief note on the subject would be of interest.

The mountain park under consideration is a small glaciated valley in the Rocky Mountains at an altitude of about 9000 feet. Thc climate is cold, the July mean being about 56° F. Most of the park has a coarse soil of decomposed granite. Upon this is a xerophytic grassland vegetation. In certain places, however, particularly at points along the margins of creeks or ponds and in glacial sinks, a mesophytic grassland develops. This may be spoken of as "meadow." Here the soil has a considerable amount of humus derived from washings of adjacent slopes. Of the 19 quadrats, 17 are in dry grassland and 2 in meadow.

The data presented (tables I and II) are arranged according to time of year. Although the observations were made in two seasons, no doubt they show normal conditions, since both years had about the usual precipitation and were not greatly different in temperatures.

TABLE I

PERCENTAGE OF BARE GROUND IN DRY GRASSLAND

| Quadrat | May 30, 1912 | June 12, 1913 | June 28, 1913 | July 12, 1913 | July 26, 1913 | Sept. 1, 1912 |
|---|---|---|---|---|---|---|
| I | 75 | 60 | ..... | ..... | 30 | 36 |
| II | 60 | 49 | ..... | ..... | 33 | 53 |
| III | 63 | 42 | ..... | ..... | 50 | 46 |
| IV | 50 | 20 | ..... | ..... | 13 | 20 |
| V | 60 | 55 | ..... | ..... | 20 | 40 |
| VI | 60 | 50 | ..... | ..... | 28 | 33 |
| VII | 50 | 38 | ..... | ..... | 20 | 28 |
| VIII | 70 | 55 | 25 | 30 | 45 | 30 |
| IX | 64 | 30 | 25 | 15 | 20 | 30 |
| X | 63 | 25 | 20 | 20 | 10 | 20 |
| XI | 80 | 75 | 50 | 32 | 50 | 40 |
| XII | 50 | ..... | 20 | ..... | 15 | 25 |
| XII (*a*) | 60 | 40 | 20 | 10 | 15 | 20 |
| XIII | 50 | ..... | 10 | ..... | 20 | 25 |
| XIV | 60 | ..... | 20 | 25 | ..... | ..... |
| XV | 50 | ..... | 20 | ..... | 25 | 50 |
| XVI | 60 | ..... | 10 | ..... | ..... | 30 |
| Average | 60 | 45 | 22 | 22 | 26 | 33 |

[2] It may be well to state that a new library card was taken each time for every quadrat study. The data were not tabulated or even looked at until the close of 1913, lest the judgment of the observer at any time might be influenced by earlier records.

The figures of 60 per cent bare ground for May 30 in table I should not be understood to mean that the other 40 per cent is fresh growth. Indeed, there is almost nothing green at that time of year. The vegetation cover is made up of the dry grasses, sedges, and flowering herbs of the previous summer. By July 1, however, the new growth has reached its maximum. This rapid development is necessitated by the shortness of the growing season. Only such plants as can mature quickly are able to exist under the rigorous climatic conditions.

TABLE II

PERCENTAGE OF BARE GROUND IN MEADOW GRASSLAND

| Quadrat | May 30, 1912 | June 12, 1913 | June 28, 1913 | July 12, 1913 | July 26, 1913 | Sept. 1, 1912 |
|---|---|---|---|---|---|---|
| XI (*a*) | 45 | 20 | 0 | 0 | 0 | 10 |
| XIII (*a*) | 25 | ..... | 20 | 10 | 10 | 10 |
| Average | 35 | 20 | 10 | 5 | 5 | 10 |

In the dry grassland, as will be seen from table I, there is a considerable proportion of bare ground, even at the height of the season in early July. The meadow association, on the other hand, shows little or no vacant space at that time. The differences depend chiefly on the texture of the soil, the finer grained material of the meadow holding more moisture and permitting more luxuriant growth.

It is interesting to note that when introduced weeds get a foothold in the region they do not become established in the open association of dry grassland, but in the already closely grown meadow.—FRANCIS RAMALEY, *University of Colorado, Boulder, Colo.*

## THE OXIDASES OF ACID TISSUES

According to recent theories, the oxidases play an essential part in respiration. If these theories are correct, it follows that oxidases must be present in all organisms. But a serious difficulty is encountered in the fact that many plants have been reported to be free from oxidases. If this is really the case, it would seem that these theories must be abandoned, or at least greatly modified.

It appeared to the writer that in view of the importance of the subject a fresh investigation was needed. From the results of the writer's experiments it is evident that in many cases the reported absence of oxidases is to be ascribed to faulty methods of investigation. The

method which has been almost exclusively used is to express the juice or to make watery extracts of the ground or minced tissue. The ability of these extracts to accelerate the oxidation of such reagents as gum guaiac, α-napthol, hydrochinone, etc., has been taken as an indication of the presence of oxidases in the respective tissues.

The extracts which appear to be without oxidases when tested in this manner have, in the majority of cases, a distinct acid reaction. This fact was noted by CLARK[3] in summarizing the results of his own experiments. He concluded that extracts having an acidity such that 10 cc. required 8 cc. 0.1 N, KOH to neutralize to phenolphthalein were without oxidases.

The writer, proceeding in a similar manner with a considerable number of acid tissues, including several not previously tested, obtained results comparable with those reported by CLARK. He then made a series of special tests on *Citrus* fruits, probably the most acid of tissues, using a different method of examination, which seems to throw light on the general condition in acid tissues. Lemons, oranges, grape fruit, and kumquats were tried. In these fruits the juice is contained in long and enlarged sacs attached to the inside of the carpel walls. From this point of attachment they extend into the carpel, giving it the familiar honeycomb-like appearance. These sacs may be readily separated from the carpels and from one another without injuring their skin, which consists of 2–10 layers of cells. In this condition, when placed in a solution of gum guaiac, α-napthol, sodium selenite, or similar reagents, along with hydrogen peroxide, the surface of the hairs soon becomes covered with the colored compound resulting from the oxidation of the reagent. This indicates the presence of oxidases (or, according to the terminology of BACH and CHODAT, peroxidases, since hydrogen peroxide was required) in the cells of the sacs which contain the juice.

It is thus evident that *Citrus* fruits have normal oxidases in their acid tissues. It is also evident that these oxidases are protected in some manner from the action of the acid which at this concentration effectually inhibits the action of oxidases. It seems to the writer that this protection may be afforded by a semipermeable surface (the plasma membrane or cell walls similar to the cell walls of barley seed, which are impermeable to acid) through which the acid is unable to pass. When the tissue is ground, previous to pressing out the juice, the structure which separates the acid from the ferment is destroyed, so that the action of the latter is inhibited. That these membranes are not normally

[3] CLARK, Torreya 11:101. 1911.

permeable to acid is shown by the fact that seeds[4] of lemons (which are separated from the acid by the walls of the sacs) frequently germinate while still in the carpels, though they will not germinate in lemon juice several times diluted.

It seems probable that this condition is a general one in acid tissues. The acid and ferment are separated in the tissue probably in a variety of ways, but the grinding destroys the separating surface, bringing acid and ferment in contact and inhibiting the action of the latter.

The general effects of acids and alkalies on oxidase ferments are now under investigation and will be reported on later.—G. B. REED, *Laboratory of Plant Physiology, Harvard University.*

## THE TYPE SPECIES OF DANTHONIA

In a recent paper,[5] Professor A. S. HITCHCOCK attempts to show that the type species of *Danthonia* DC. is *Avena spicata* L. instead of *Festuca decumbens* L. If his arguments are to stand, stability in nomenclature will be an impossibility, for they are based purely on personal opinion. If a case in which there is doubt as to the type of a genus is to be decided on an interpretation of what the author may have thought most representative, it will never be disposed of, for this decision may vary with each succeeding systematist who faces the problem.

As emphasized at the Vienna Congress, one of the fundamental points in nomenclature[6] "is to aim at fixity of names," and "in the absence of rule" (by which to bring this about) "established custom becomes law." However logical, therefore, Professor HITCHCOCK'S decision as to the type of *Danthonia* may be, it is essential for the sake of nomenclatural stability that the accepted custom of selecting the first species described (when the type is not indicated) be adhered to. Otherwise, as pointed out by Professor HITCHCOCK, some other botanist may say, "I favor selecting" *D. provincialis* DC. as the type.

Now *Festuca decumbens* is not only the first species described, but Professor HITCHCOCK fails to show that DE CANDOLLE did not consider it completely congeneric with his *D. provincialis*. It is provided with an awn, even though rudimentary, and, to quote, "it is evident that the author considered the awn to be one of the important distinguishing characters of his new genus." DE CANDOLLE'S suggestion that *Avena*

[4] The seeds of all the *Citrus* fruits examined showed an abundance of oxidases.

[5] The type species of *Danthonia*. BOT. GAZ. **57**:328. 1914.

[6] Vienna Rules, 35. 1905.

*spicata* should be included surely does not indicate that he regarded it as *more* representative of his *Danthonia* than *decumbens*, since his generic description provides for the latter ("awn sometimes long, sometimes rudimentary").

The adoption of *Avena spicata* L. as the type, therefore, is seen to be purely arbitrary, since such action is based on the present interpretation of the genus.—AVEN NELSON and J. FRANCIS MACBRIDE.

## MATURATION IN VICIA

(PRELIMINARY NOTE)

The following preliminary note summarizes the results so far obtained in a study which has been temporarily interrupted. Although many details remain to be worked out, the following points seem clear.

In the somatic cells of *Vicia Faba* there are twelve chromosomes; two of them are about twice as long as the other ten. How this size difference arose is not known, but there is some reason to believe that each long chromosome may have been formed originally by the coherence of two ordinary ones.

In the early prophases of the heterotypic mitosis in the pollen mother cells, the chromosomes take the form of long slender threads (leptonema), which become paired side by side (zygonema). These double threads shorten and thicken (pachynema), the association of the two members of each pair becoming very intimate. The nature of this union has yet to be determined. Synizesis occurs during these prophase stages as a natural phenomenon.

At diakinesis there are six gemini; one of them is about twice as large as the other five, showing that the two large chromosomes seen in the somatic cells have paired with each other. At the first maturation division the members of each pair pass to opposite poles, bringing about the reduction. In the second, or homeotypic, mitosis all the chromosomes divide longitudinally, so that each microspore receives six chromosomes, five short and one long.

The megaspore mother cell has not been examined, but in the light of the above data on somatic and pollen cells it seems probable that similar phenomena occur in the maturation of the megaspore.

The results here recorded are of special interest in that they furnish further evidence in favor of the theory that the two chromosomes which pair and separate at the first maturation division come one from each parent, and are in some sense homologous.—LESTER W. SHARP, *University of Chicago*.

# CURRENT LITERATURE

## BOOK REVIEWS

### Irritability of plants

The experimentation described in BOSE's new volume[1] is marked by excellence of methods and execution. The presentation is in direct, clear, and in many places elegant English. The findings are rendered quickly available by comprehensive but concise summaries at the end of each chapter and at the end of the volume. One regrets that this excellent work is marred here and there by oldness of viewpoint and lack of knowledge of physiological literature.

In his older researches BOSE used the optical lever with the photographic method of recording. This made the taking of automatic records tedious, with the possibility of relatively few records during the active season of the plant. It also forced experimentation in an abnormal condition for the plant, namely, continual darkness. The resistance offered by the recording apparatus, both because of weight of the arm and because of the friction of the writing point on the black surface, was another difficulty in the taking of automatic records. The author has overcome all of these difficulties by his new and ingenious resonant and oscillating recorders. He has designed arms that are about 0.01 as heavy as those used with the muscle nerve, and the oscillating or resonant recorder gives a series of dots on the black surface rather than a continuous line. This reduces the friction to a very small fraction of that of the continuous record. In the *Desmodium* leaflet the author has shown that a continuous line gives a great reduction in the magnitude of the movement, while none occurs with the new recorder. His new apparatus also makes possible the recording of periods as short as 0.001 of a second. One is convinced that BOSE is reaching the optimum of accuracy and delicacy in the matter of automatic records of plant response, and this is much needed, for many fewer such records exist for plants than for animals.

This excellent experimentation results in a number of things of interest to plant physiologists; in some cases confirming views already held; in others showing prevailing views at error; and in still others giving exact determinations of physiological critical time periods. BOSE shows that the sum of stimulus law holds for a number of stimuli, as has been shown to be the case for geotropism, heliotropism, etc. In subtonic condition of an organ the stimulus itself renders the organ more excitable to the same stimulus. He has apparently established beyond doubt that conduction of a stimulus in the

[1] BOSE, J. C., Researches on irritability of plants. xxxiv+376. *figs. 190.* London: Longmans, Green & Co. 1913.

petiole of *Mimosa* cannot occur over a zone that is dead or in a state of rigor, contrary to the claims of HABERLANDT and PFEFFER. As BOSE puts it, conduction of stimuli in this organ is a true "excitatory" or "physiological" process. His statement that plant physiologists universally hold that conduction of stimuli in plants is "hydromechanical" shows that he has entirely overlooked the generally accepted and much quoted classic of FITTING (1907) on the conduction of the light stimulus in the coleoptile of *Avena*. The author's findings in *Mimosa* do not, contrary to his statement, disprove a universally held view in plant physiology, but they merely show that the conduction of stimuli in this form is consistent with that in *Avena*, which probably represents the general situation for plants. He has shown that PFLUGER's laws of polar excitation in animal tissues hold for plant tissues, and supplements these by two more laws that hold for strong currents. This is only one of many illustrations that BOSE brings forward of fundamental likenesses in stimulus effects and response in plants and animals. Records are given of very accurate determinations of latent periods (the time elapsing between application of the stimulus to the pulvinus and the beginning of movement) in the leaves of *Mimosa* and other motile leaves, as well as exact measurements of the rate of conductivity of stimuli. In the primary pulvinus of *Mimosa* the shortest latent period found was 0.06 second, and the average 0.1 second. In subtonic but not in optimum condition of the organ, increased strength of the stimulus shortens, while in general fatigue prolongs, and a rise in temperature shortens this period. The maximum rate of conduction was that found in the petiole of *Mimosa;* it was 30 mm. per second. Conditions that shorten the latent period also increase the rate of conduction. In subtonic but not in optimum condition of the organ, previous application of the stimulus hastens the rate of conduction. Conduction takes place in both directions, but not always at the same rate. In *Biophytum*, for instance, the velocity is greater in the centrifugal than in the centripetal direction.

BOSE emphasizes that conduction of the stimulus proper is accompanied by a wave of contraction and of galvanometric negativity. This is contrasted with the wave of galvanometric positivity which passes over a stimulated non-living object or as a secondary effect of stimulation in the living organ, and which travels much faster than the primary stimulus effect. The author emphasizes this distinction between true "excitatory" or "physiological" conduction, accompanied by contraction and galvanometric negativity, and the "physical" conduction, marked by galvanometric positivity. This is good, but physiology is beyond the stage of stopping with such distinctions. It now recognizes that the effect and conduction of stimuli proper in the organism are physical also, undoubtedly more complex than the behavior in the non-living, but physical nevertheless. Two groups of facts, each in part and both together, probably fully explain the physics of the contractile wave with its galvanometric negativity. One group of workers[2] has produced much

[2] Science N.S. **37**:959–974. 1913.

evidence that the living cells of the organism are surrounded by semipermeable membranes with a difference in electrical potential outside and inside the membrane, and that any stimulus causes an increase of permeability with its necessary contractile and electrical effects. Again, the behavior of colloids in the form of gels leads one to believe that hydration-changes accompany response to stimuli in the protoplasm, which always involve contractile and electrical effects. These are probably the physical factors entering into BOSE's "physiological" response and conduction. With death (coagulation of the colloids) both the membranes and the colloids disappear, and with them the "physiological" response.

Many statements in the book show a lack of familiarity with the anatomy and physiology of plants. The author speaks repeatedly of depleted energy in the pulvinus of *Desmodium*, or of its receiving energy from without. This is rather vague in the light of what we know of the energy relations of plants. He applies systole and diastole to the pulvinus as he does to the heart, and compares the petiole of *Mimosa* with the nerve and the pulvinus with the muscle of a nerve muscle preparation. Both of these statements are made in spite of the fundamental differences in structure and function. The death point for plant cells is fixed, as if mysteriously, at 60° C., and death from a lower temperature is spoken of as being very different in nature. BOSE fails to recognize that death at supramaximal temperatures is a matter of coagulation of certain proteins, and that at any supramaximal temperature the time of its application is a function in the process. At temperatures lower than 60° C. the time must be longer. The reason that 60° C., rather than a lower or higher temperature, is found to be the death temperature in a given species, is because of his method of heating, namely, 1° C. rise in temperature per minute. A slower heating would give a lower death point, and a faster one a higher one. The reason for the constancy of this temperature for the different species studied is not so clear. BOSE should have been acquainted with LEPESCHKIN'S[3] work on this subject. It is of some interest that the death temperature is lowered by fatigue to 37° C., and by copper sulphate to 42° C. This emphasizes again the marked lability of certain cell proteins, and reminds one of LEPESCHKIN'S results showing that they are coagulable even by pressure.

BOSE is adding much to the subject of plant response, but his excellent methods could add much more if they were applied in the light of our modern knowledge of the physics and chemistry of living cells and of plant response in general.—WILLIAM CROCKER.

### Biology and capillary analysis of enzymes

GRÜSS[4] has brought together in book form the results of his work on enzymes, which has extended over a period of several years. Some of his con-

[3] Ber. Deutsch. Bot. Gesells. **30**:703–714. 1913.

[4] GRÜSS, J., Biologie und Kapillaranalyse der Enzyme. 8vo. pp. vi+227. *col. pls. 2. figs. 58.* Berlin: Gebrüder Borntraeger. 1912. *M.* 16.

clusions are a decided departure from the usual conceptions regarding enzyme action. Probably the most striking departure is the assumption that different enzyme actions may be attributed to different atomic groups of the same enzyme; for example, malt diastase is also capable of cytase and peroxidase action. Many of the data upon which his conclusions are based are drawn from capillary analyses. This method for the separation of soluble substances the author has very ingeniously modified and elaborated for his particular purposes. With modifications excluded in special instances and stripped of details, the general method may be described as follows: A few drops of the enzyme mixture to be analyzed are placed upon filter paper. By capillary attraction a circular field is formed in which the dissolved substances arrange themselves in more or less distinct zones. In the case of colloidal substances, a greater spreading and separation is effected if a water ring is first placed upon the filter paper. Diffusion forces then come into play as well as capillary attraction. Diffusion is rendered more active if the solution to be analyzed contains a little glycerine. After spreading ceases, the capillary field is cut into sectors and each treated with a different reagent which gives a color reaction for a specific enzyme. The diastase test, for example, is carried out by placing firmly a sector of the capillary field upon a similar piece of filter paper moistened with a solution of soluble starch. After the diastase present in the sector of the capillary field has had sufficient time to hydrolyze the starch, the starch paper is treated with iodine solution. The presence and extent of the diastase in the field are then shown by a white area bordered by the blue-violet color of the starch iodine reaction. For peroxidase a sector is treated with guajak+$H_2O_2$; for oxydase, violamin is employed, etc. The treated sectors are all brought together to form the "chromogram," which shows the distribution or relative spreading of the different enzymes in the capillary field. If the same area is capable of more than one enzyme action, the author believes that it is due to a complex enzyme and not to the coexistence of different independent enzymes. He also believes that he has further confirmed his theory by showing that certain complex enzyme actions of capillary analysis appear also simultaneously in germination and are simultaneously destroyed by a given treatment of the enzyme preparation.

The enzymes of various embryos and endosperms, potato tubers, fungi, yeast, and latex were extensively studied with the aid of the capillary method, together with microchemical tests. The following are a few of the author's most important biological inductions: The enzymes and their anti-enzymes form systems in which the members oscillate around an equilibrium position. Upon these systems, which deserve the designation *Träger des Lebens*, rests the synthesis and disintegration of the cell substances. The most important system is the group of oxidizing enzymes: oxidase+peroxidase+antioxidase (hydrogenase). Antioxidase is simply a general term indicating any inhibition of an oxidase action. The antioxidase function is probably exercised by many different kinds of substances which are not all of an enzyme nature, since

in many cases they are changed by their own activity and are not destroyed by hot alcohol. The enzyme nature of hydrogenase, however, is not to be doubted. It performs its function by liberating nascent hydrogen which unites with the oxygen carried by the oxidase to form water or hydroxyl groups; the latter can be employed for the formation of metabolic products. By these formations the oxidation of other substances is prevented. "Reductase" is a *contradictio in adjecto*. "Catalase" is merely a term to designate the decomposition of $H_2O_2$, and its place in the category of enzymes is extremely hypothetical. Amylocoagulase is the anti-enzyme of diastase in the sense that its action is just the opposite of that of diastase. The coagulase may be entirely obscured by the action of diastase, but it cannot be reversed. Cytocoagulase is another synthetic enzyme which acts in opposition to cytase.

The chief value of the book lies in the mass of experimental details and the ingenious methods devised for the study of enzymes. It will find its greatest usefulness among those especially interested in enzymes, as the treatment is rather technical.—CHAS. O. APPLEMAN.

**Field manual of trees**

An addition to the already numerous tree manuals has come[5] in the form of a thin pocket volume, 4.5 by 7 inches, bound in flexible leather, making it particularly convenient for field use. It is designed to include all the native and many of the introduced species north of Virginia and Kentucky and east of the prairie region. The features which recommend this manual are the numerous keys, all of which appear to be accurate and rather simple. They include separate ones for the genera based on (1) summer condition, (2) winter condition, (3) flowers, and (4) fruit, as well as keys for species under each genus and a general classification of the wood of the trees. The descriptions of the species seem to be good, but it is certainly unfortunate that such well known trees as the black and choke cherry should appear under such scientific names as *Prunus virginiana* and *P. nana* respectively, and that the flowering dogwood should be removed from the genus *Cornus*. Whatever may be the arguments of the systematist for such a course, it is certainly likely to lead to confusion, especially in a manual where no synonyms are given. One also misses the illustrations which have been a prominent feature of many recent volumes on trees.—GEO. D. FULLER.

## MINOR NOTICES

**Botanical researches of the Carnegie Institution.**—The annual report[6] for the past year gives an idea of the various lines of research completed and

[5] SCHAFFNER, JOHN H., Field manual of trees, including southern Canada and the northern United States to the southern boundary of Virginia, Kentucky, and Missouri, westward to the limits of the prairie. Columbus (Ohio): R. G. Adams & Co. 1914. 16mo. pp. 154. Cloth $1.25. Limp leather $1.75.

[6] MACDOUGAL, D. T., Annual report of the director of the department of botanical research. Carnegie Institution of Washington, Year Book No. 12 for 1913:57–87. 1914.

in progress at the Desert Laboratory of the Carnegie Institution and of the reports that are soon to appear. The activities are so numerous and the scientific staff so large that all cannot be mentioned in a brief notice, but some are especially worthy of note.

Studies upon the Salton Sea by MACDOUGAL and several of his associates since 1907 have been very extensive, and include investigations of the soil and rock deposits, yearly analyses of the water as the sea continued to dry up, studies of the action of this water upon vegetable tissues, of the destruction of the flora upon the submerged areas, and of the revegetation of the sterilized islands and beaches. These investigations have given remarkable results from the unique phenomena of this semi-arid region. A few of the reports have appeared and more are to be published very soon. The modification of plants under the specialized conditions of the Salton area are being studied, as well as the effects of various climatic complexes. W. A. CANNON is continuing his investigations of the root characters of desert plants and has also extended his activities to include the roots of trees grown in the coastal climate of California. FORREST SHREVE and his associates have made critical studies of transpirational behavior in many very diverse plants, and also of growth rate and winter temperature in the Santa Catalina Mountains. H. A. SPOEHR has been investigating the photolytic effect of the blue-violet rays and their variations in polar radiation; while B. E. LIVINGSTON, with several assistants, has been making exact studies of the water relations of plants, including both the aerial and the soil conditions. The fruit development of the Cactaceae has been investigated by D. S. JOHNSON and the relationships and distribution of the same family by N. L. BRITTON and J. N. ROSE.—GEO. D. FULLER.

**Annals of the Bolus Herbarium.**—The first South African journal of botany has just made its appearance under the above title. The editor is Professor PEARSON of the South African College. The first number of the journal contains 40 pages, and it is announced that two parts will probably appear each year, and that four parts will constitute a volume. The reasons for a South African journal of botany are given, and seem to be well taken. The field for botanical investigation in South Africa is very large and but sparsely occupied, and the number of those directly or indirectly interested in botanical work is increasing. The new journal, as its name implies, will be mainly concerned with the botanical work inspired by Dr. BOLUS and with investigations conducted in connection with the Bolus Herbarium. This means that its field will be chiefly the taxonomy, ecology, plant geography, and economic botany of South Africa. There will also be included articles that may prove of assistance to those engaged in teaching botany. In a certain sense the journal will also be an organ of the newly established National Botanic Garden.

The first number contains the following papers: "On the flora of the Great Karasberg," with an introduction by PEARSON giving an account of the region, and a list of plants by F. and L. BOLUS and R. GLOVER; "Novitates

Africanae," in which a new genus (*Pillansia*) of Iridaceae is described by L. BOLUS; and "Key to the flora of the Cape Peninsula," by F. and L. BOLUS. —J. M. C.

**Annals of the Missouri Botanical Garden.**—The present year has been prolific in the appearance of new botanical journals. To the *American Journal of Botany* and the *Annals of the Bolus Herbarium* is now added the *Annals of the Missouri Botanical Garden*. The new journal is a quarterly, the first number being dated March 1914. The journal will provide for the printing of scientific papers which formerly constituted a large part of the annual report of the Missouri Botanical Garden. It will contain only scientific contributions from members of the staff of the Garden, from the faculty and graduate students of the Washington University, and from visiting botanists doing all or part of their work at the Garden. The first number contains the following papers: "The effect of surface films and dusts on the rate of transpiration," by B. M. DUGGAR and J. S. COOLEY; "Some pure culture methods in the algae," by JACOB R. SCHRAMM; "The identification of the most characteristic salivary organism and its relation to the pollution of air," by AUGUST C. NOLTE; "The *Polyporaceae* of Ohio," by L. O. OVERHOLTS.—J. M. C.

**The fresh-water flora of Germany, Austria, and Switzerland.**—Part 1 of this series of brochures has appeared.[7] The five previous parts have been noticed in this journal.[8] The present part completes the flagellates, the other groups of which were presented by PASCHER and LEMMERMANN in part 2. The compact size, excellent illustrations, and well considered analytic keys continue to be features of this excellent work.—J. M. C.

## NOTES FOR STUDENTS

**Color inheritance.**—Continuing his excellent studies on *Melandrium* (*Lychnis*), SHULL[9] has made a great advance in our knowledge of the inheritance of leaf pigments of the chlorophyll and the carotin-xanthophyll groups. With his characteristic care, the author came to his conclusions only after a very large number of hybrids properly synthesized for the tests desired had been made. The color wheel was used as an aid to the classification of the individuals wherever it was deemed necessary. BAUR's discovery of a general factor for chlorophyll formation (*Z*), without which plants are free from chlorophyll, is confirmed; but his idea that the gene *Z* produces yellow pigment is not supported. Assuming the presence of unanalyzed genes *XX*, then typical

[7] PASCHER, A., Die Süsswasser-Flora, Deutschlands, Österreichs, und der Schweiz. Part 1. Flagellatae, by A. PASCHER and E. LEMMERMANN. pp. 138. *figs. 252*. Jena: Gustav Fischer. 1914.

[8] BOT. GAZ. **56**:233. 1913; **57**:335. 1914.

[9] SHULL, G. H., Über die Vererbung der Blattfarbe bei *Melandrium*. Ber. Deutsch. Bot. Gesells. **31**:40–80. 1914.

dark green plants of *Melandrium* are *XXZZYYNN*. This was shown by crosses between two light green types, *chlorina* and *pallida*. The $F_1$ generation was the typical dark green, while the $F_2$ generation consisted of dark green and light green in the ratio 9:7. Among the light green individuals both *chlorina* and *pallida* plants could be recognized, but just what characters the plants with the formula *XXZZyynn* possessed was not determined.

Three cases of non-mendelizing leaf variegation are also described:

1. This case of variegation was a chimera made up of typical dark green and of pure white areas. Seed from green branches or from white branches produced progeny exactly like the mother, no matter what characters were possessed by the male parent.

2. These plants are called *chlorinomaculata*, because they are dark green spotted with the "chlorina" type of green. The transmission of their characters is not yet entirely clear. The progeny of a female plant crossed with pollen from flowers of different colored branches gave the following results: from variegated branches came green, variegated, and chlorophyll-free plants; from green branches came only green plants; and from *chlorina* branches came only chlorophyll-free plants.

3. These plants were of the yellowish *aurea* type. They were crossed with many other forms, but the results are somewhat complex, and the author does not commit himself definitely on their analysis. He thinks that possibly this may be a case of infectious chlorosis. He says: "While chlorosis of *Abutilon* and other Malvaceae is transmitted neither through the male nor the female gametes, this *aurea* character is carried by a part of the gametes of both kinds." It seems to the reviewer that if this phenomenon is indeed one of infectious chlorosis, the small number of *aurea* plants of the filial generations might easily be due to reinfection.—E. M. East.

**Statistical methods in phytogeography.**—In his attempts to obtain more exact data regarding the distribution of the various elements of alpine flora, Jaccard[10] has developed certain statistical methods that have not only revealed several interesting facts regarding the vegetation of the Alps, but promise to be equally serviceable in the investigation of other areas. Having made a census of the areas to be compared, in this instance similarly situated localities of approximately the same area in various parts of the Alps, he applies for the analysis of his results his *coefficient of community* (C.c.), that is,

$$\frac{\text{No. of species common to two districts} \times 100}{\text{Total no. of species in the two districts}} = \text{C.c.}$$

For alpine meadows at an altitude of 1900 m., several interesting results were manifest, such as: (1) the fact that the value of C.c. does not depend upon floral richness, but upon the ecological characters of the areas studied; (2) the

[10] Jaccard, Paul, The distribution of the flora in the alpine zone. New Phytol. 11:37–50. 1912.

alpine flora is extremely diverse in floristic composition; (3) the rare species are most numerous and the common species least numerous (this does not apply to number of individuals); and (4) the C.c. is generally higher for contiguous than for distant areas. This last result is strikingly demonstrated in the study of the vegetation upon some alpine gravel areas[11] of the Alps. In this latest report JACCARD also makes extensive use of his generic coefficient (*coefficient générique;* C.g.), that is,

$$\frac{\text{No. of genera} \times 100}{\text{No. of species}} = \text{C.g.},$$

with instructive results. This coefficient is shown by studies of both alpine and dune floras to vary inversely with the variety of ecological conditions in the areas compared, and hence in alpine areas the value of C.g. increases with altitude, while in some dune areas of Belgium, the C.g. is greatest (100) under the excessive and narrow ecological limits of the moving dunes, and least (73) under the more varied ecological conditions of the pannes.

These analyses lead the author to the following among other conclusions: (1) The distribution of plants (at least in the alpine zone) is a resultant of the combined action of three orders of factors; (*a*) ecological, (*b*) biological, or degree of adaptation, and (*c*) sociological, or competition between species; and (2) the action of these factors has resulted in (*a*) an eliminative selection of species, and (*b*) a distributive selection determining the number of individuals and the nature of associated species.—GEO. D. FULLER.

**Tetraspore formation in Nitophyllum.**—Since it has been shown that in *Polysiphonia* and *Dictyota* the reduction of chromosomes occurs during the formation of tetraspores from the tetraspore mother cell, it is natural to inquire what cytological conditions obtain during tetraspore formation in those red algae which have multinucleate cells. One might guess that the tetraspore mother cell is uninucleate, or that it is multinucleate and all the nuclei except one disorganize. Soon after YAMANOUCHI'S paper on *Polysiphonia* appeared, SVEDELIUS examined *Martensia,* one of the Delesseriaceae. The tetraspore mother cell is multinucleate and, as the cell enlarges, the nuclei multiply until there are about fifty. Then all but one disorganize, and four tetraspores are formed. The fixing did not allow a detailed cytological study. Recently, however, SVEDELIUS[12] secured well fixed material of *Nitophyllum punctatum,* a member of the same family, and succeeded in working out the cytological situation. The thallus is prevailingly one cell thick, but when tetraspores are so formed, it becomes three or four cells thick. The tetraspore mother

[11] JACCARD, P., Étude comparative de la distribution florale dans quelques formations terrestres et aquatiques. Rev. Gén. Botanique **26**:5–21, 49–78. 1914.

[12] SVEDELIUS, N., Über die Tetradenteilung in den vielkernigen Tetrasporangiumanlagen bei *Nitophyllum punctatum.* Ber. Deutsch. Bot. Gesells. **32**:48–57. *pl.* 1. 1914.

cell has several nuclei, and nuclear division continues after the mother cell has become recognizable, but the number of nuclei seldom exceeds a dozen. In the vegetative mitoses the chromosomes are organized directly from the reticulum, but in the tetraspore mother cell the formation of chromosomes is preceded by a typical spirem stage. After the number of nuclei reaches about a dozen, some begin to disorganize, but several may develop spirems and continue up to a typical metaphase of the heterotypic mitosis; then one nucleus continues and all the rest disorganize. The details of the division of the successful nucleus and the organization of tetraspores agrees fully with the account given by YAMANOUCHI for *Polysiphonia*.—CHARLES J. CHAMBERLAIN.

**Distribution and development of an Ohio flora.**—A region in southern Ohio designated as "Sugar Grove" and situated at the end of a long lobe of Merriam's Alleghenian floral area, is regarded by GRIGGS[13] as remarkable on account of its being the meeting place of many very diverse floras. He has mapped six such groups, consisting of (1) Alleghenian plants, 39 species; (2) Appalachian plants (northern), 14 species; (3) Appalachian plants (southern), 12 species; (4) Carolinian plants, 12 species; (5) Mississippian plants, 15 species, and (6) northern plants, 9 species, and finds Sugar Grove upon extreme limits of each group. The data for establishing the range of these 121 plants are scanty, as the author admits, but that many of these species are near the limit of their distribution seems quite evident. This has led to an investigation of the behavior of many species at the edges of their range,[14] and almost every possible phase of behavior is found to be exhibited. So varied appear the responses that it would seem difficult to draw any general conclusions, although the author decides that the limits of species reaching the edges of their ranges near Sugar Grove are not fixed, but are changing, and that plants of boreal affinity are apparently being displaced by others from the west and south, a continuation of the floristic movements following the glacial period. *Tsuga canadensis* is cited as an example of such movements, and GRIGGS asserts that it is now found in southern Ohio only because it has not been completely displaced by the post-glacial flora, and occupies its habitats simply because within them the invading hardwood forest has not had so good an opportunity to gain a foothold as elsewhere. This explanation seems plausible, but the reviewer cannot regard the case as proved.—GEO. D. FULLER.

**Prothallium of Equisetum.**—KASHYAP[15] has investigated the prothallium of *Equisetum debile* as it grows in abundance in the vicinity of Lahore, India.

[13] GRIGGS, R. F., Observations on the geographical composition of the Sugar Grove flora. Bull. Torr. Bot. Club **40**:487–499. 1913.

[14] GRIGGS, R. F., Observations on the behavior of some species at the edges of their ranges. Bull. Torr. Bot. Club **41**:25–49. 1914.

[15] KASHYAP, SHIV R., The structure and development of the prothallium of *Equisetum debile* Roxb. Ann. Botany **28**:163–181. *figs. 45.* 1914.

This first investigated Asiatic species proves to be of great interest, as the following summary of results will show. The prothallium is exceedingly variable in its early stages, and of special interest is the occasional occurrence of a "primary tubercle" comparable to that of *Lycopodium cernuum*. The lobes of the prothallium are always erect and very close together, both in nature and in a darkened room, so that this upright position holds no relation to the amount of light. One of the striking features of the prothallium is its radial symmetry, which disposes of the claim that the fundamental difference between the prothallium of *Lycopodium* and of *Equisetum* is that the latter is not radial, but dorsiventral. The prothallium of this species also proves to be much larger than the largest that have yet been found in the genus. There are no male prothallia, but sometimes prothallia do not produce antheridia, and therefore are female. The antheridia resemble those of *Lycopodium* in position, general structure, and paraphyses. The archegonium has a single neck canal cell, which is also a feature of resemblance to *Lycopodium cernuum*. The author reaches the general conclusion that there is a clear affinity with the prothallium of *Lycopodium cernuum*, and that there is no more difference between the two prothallia than is already known to occur among the species of *Lycopodium*.—J. M. C.

**The embryogeny of Balanophora.**—The researches of TREUB and LOTSY on the embryogeny of the Balanophoraceae are well known. In *Balanophora* they found the four nuclei in the antipodal end of the sac, and also the synergids and egg degenerating as soon as the sac reached the fertilization stage; the remaining micropylar polar nucleus gave rise to a cellular endosperm, from one of whose cells the embryo developed.

ERNST'S studies on the embryogeny of saprophytic forms led him to suspect that there might be a simpler explanation of the origin of the embryo of *Balanophora*. A reinvestigation[16] confirmed the previous accounts of the origin and development of the embryo sac, the degeneration of the antipodals and the synergids, and the formation of a cellular endosperm from the micropylar antipodal; but it also showed that the embryo is developed from the egg. The development, however, begins late, after the egg is surrounded by cellular endosperm, and it was this behavior which misled both TREUB and LOTSY. There is no fertilization in either *Balanophora globosa* or *B. elongata*. Both are parthenogenetic. The development of the sac shows the diploid number of chromosomes.—CHARLES J. CHAMBERLAIN.

**Experimentation in plant geography.**—MASSART[17] has given emphasis to the fact that plant geography has hardly kept abreast of other branches of

[16] ERNST, A., Embryobildung bei *Balanophora*. Flora **106**:129–158. *pls. 1, 2.* 1913.

[17] MASSART, J., Le role de l'éxperimentation en géographie botanique. Rec. Instit. Bot. Léo Errera **9**:68–90. 1913.

biological science on account of the lack of experimental methods of investigation, and he has consequently undertaken to indicate certain classes of problems which are demanding experimental solution. Certain species, for example, occupy habitats so diverse that there seems legitimate ground for doubting whether the different forms are specifically identical. Amphibious species of *Polygonum* furnish striking examples of form variation and specific relations only to be determined by extensive experimental cultures. Here he recommends that "accommodations" be limited to transformations of individuals in response to changed environment, and "adaptation" to those of species, the former not being hereditary, but the latter being transmitted to offspring. The author also urges the investigation by experimental methods of all examples of apparent multiplicity of origin of species, and of natural hybrids and mutations.—GEO. D. FULLER.

**Vascular anatomy of the Cycadophytes.**—Miss BANCROFT[18] has traversed the evidence of the relationship between the vascular anatomy of the Cycadofilicales and the Cycadales. The particular vascular type of Cycadofilicales from which the cycad line has developed is the problem to which at least three answers have been given. SCOTT favors origin from the *Lyginodendron* type; WORSDELL from the *Medullosa* type; and Miss DE FRAINE from a situation represented by *Sutcliffia*, which rather mediates between the two preceding views. Miss BANCROFT has done good service in bringing the whole evidence of this critical situation together, and has reached the conclusion that the *Lyginodendron* and *Medullosa* types have arisen from a common stock, and that from this stock the cycad line has arisen, touching very close to the simpler forms of the *Medullosa* group. When competent opinion differs as to the particular origin of a given group, it will generally be found that the dispute can be arbitrated by referring all of the groups to a common origin.—J. M. C.

**Leaf and root.**—Many find difficulty in identifying a stem in some vascular plants. CHAUVEAUD[19] diagrams the first leaf and root of *Ceratopteris thalictroides* and calls the combination a *phyllorhiza;* the second leaf with its root constitutes the second *phyllorhiza*, etc. The fused portions are commonly called the stem. *Cordyline anstralis* presents a similar condition. In dicotyls, the first two phyllorhizas are not separated in time or space; they have a fused portion, the stem, and a common root. Why it should make any difference as to whether the fusion exists from the start, or occurs a little later, is not entirely clear. However, it must be admitted that even in some plants with two cotyledons, like the cycads, the two categories, leaf and root, seem sufficient for the

[18] BANCROFT, NELLIE, Pteridosperm anatomy and its relation to that of the cycads. New Phytologist **13**:1 and **2**:41–68. *figs. 20.* 1914.

[19] CHAUVEAUD, GUSTAVE, La constitution et l'évolution morphologique du corps chez les plantes vasculaires. Compt. Rend. **158**:343–346. *figs. 8.* 1914.

seedling, and that the stem category is rather artificial.—CHARLES J. CHAMBERLAIN.

**An arctic-alpine plant association.**—Upon the "snow-flush," a substratum deposited on gentle slopes or flats by streamlets of snow water and composed of fine snow-dust material, there develops a characteristic association or succession of associations, recently described by SMITH.[20] He indicates its occurrence on Ben Lawers and cites the work of others, notably that of SCHRÖTER, RÜBEL, and BROCKMANN-JEROSCH, concerning its development upon the Alps. Pioneer algae are succeeded by a thick mat of the liverwort *Anthelia juratzkana*, which gives character and name to the association. *Polytrichum* sp. follows and is succeeded by *Salix herbacea*, *Alchemilla*, *Gnaphalium*, and other alpine plants, the floristic composition of the later stages varying in different localities.—GEO. D. FULLER.

**Plant geography of the heights of Hautie.**—ALLORGE[21] has made a floristic study of a plateau 15 by 10 kilometers in area, situated northwest of Paris at the confluence of the rivers Seine and Oise. The elevation of the plateau is about 190 meters, and it exhibits a considerable diversity of soil, with comparatively natural vegetation. The associations have been segregated according to the chemical nature of the soil, and that of the calcifuges is found to be most conspicuous and to cover almost the entire top of the plateau. The regional affinities of the flora are examined and shown to be chiefly western, although the area also seems to be a rather notable meeting ground of certain northern and southern forms.—GEO. D. FULLER.

**Nitrite assimilation.**—KOSSOWICZ[22] has found that molds (*Aspergillus niger*, *Penicillium glaucum*, *Mucor Bodin*, and others) can readily assimilate nitrite when it is the only source of nitrogen. It is important that by the most delicate test (NESSLER'S method), $HN_3$ could not be detected in the cultures except in two instances, and in these only after long cultural periods (26 days). The nitrite-ion can evidently then be directly assimilated without the intermediate production of $NH_3$.—E. M. HARVEY.

**A cytological life cycle.**—In a series of diagrams based upon the life history of the fern, GRIGGS[23] presents current notions as to the behavior of chromosomes in the sporophyte and gametophyte, and also during fertilization and reduction. While the illustration should not be pressed too far, the diagram will be useful for didactic purposes.—CHARLES J. CHAMBERLAIN.

[20] SMITH, W. G., Anthelia: an arctic-alpine plant association. Scot. Bot. Rev. 1:81–89. 1912.

[21] ALLORGE, A.-PIERRE, Essai de géographie botanique des hauteurs de l'Hautie et de leurs dépendence. Rev. Gén. Botanique **25**:417–431, 472–493. 1913.

[22] KOSSOWICZ, ALEX., Nitritassimilation durch Schimmelpilze. Zeitschr. Gärungsphysiol. 3:321–326. 1914.

[23] GRIGGS, R. F., A cytological life cycle. Ohio Naturalist 13:142–145. *pl. 6*. 1913.

# GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold face** type; synonyms in *italic*.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